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July 28, 2020

Ms. Tracy Egan
Executive Director
New York State Thoroughbred Breeding and Development Fund
One Broadway Center, Suite 601
Schenectady, NY 12305

Dear Ms. Egan:

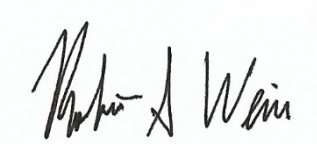
Enclosed is an electronic copy of the 2019 annual report for the Harry M. Zweig Memorial Fund for Equine Research, covering the award period of January 1, 2019 through December 31, 2019.

Included with the report are copies of the spring and fall issues of the Zweig News Capsule. We also hosted a series of research presentations highlighting equine research at Cornell on November 13, 2019 at the Cornell University College of Veterinary Medicine in Ithaca, New York. This event marked the 40th anniversary of the Zweig Fund at Cornell. Talks were given by College faculty and covered an array of equine research topics (Appendix D). A special presentation was made by alumna Lauren Schnabel, DVM '04, PhD '13, Associate Professor at North Carolina State University. The presentations were followed by a poster session and a reception attended by Zweig Committee members, faculty, scientific staff, and administrators.

Additional information about the Harry M. Zweig Memorial Fund for Equine Research can be found on the Zweig public site at https://bit.ly/zweigfundcornell.

On behalf of Cornell University, we wish to extend our appreciation for your continued support of equine research.

Sincerely,



Robert S. Weiss, Ph.D.

Professor of Molecular Genetics
Associate Dean for Research & Graduate Education

Cc: Lorin D. Warnick, PhD, Austin O. Hooey Dean of Veterinary Medicine
Ms. Jill LaBoissiere, Comptroller, NYS Thoroughbred Breeding & Development Fund
Mr. Adam Lawrence, Registrar, NYS Thoroughbred Breeding & Development Fund

Cornell University
College of Veterinary Medicine

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The Harry M. Zweig Memorial Fund for Equine Research

2019 Annual Report

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SUMMARY REPORT

The 2019 Annual Report covering the period of January 1, 2019 through December 31, 2019 is provided herein.

For this reporting period, The Harry M. Zweig Memorial Fund for Equine Research Committee awarded funding for five of fourteen submitted projects. Four of the five projects were new, first-time submissions and one was a renewal. Two of the five were recommended for partial funding. The total amount allocated for new awards for calendar year 2019 was $362,798. This report includes the “lay summaries” for the public website (Appendix A). There were also three continuation awards approved for second year funding in the amount of $262,755.

Additionally, on Wednesday, November 13, 2019, the Veterinary College hosted the 11th annual poster session and scientific talks, celebrating the collaboration between the Harry M. Zweig Memorial Fund for Equine Research and Cornell University College of Veterinary Medicine. Participants included Cornell faculty, students and scientific staff, showcasing their research to the College community and to the Zweig Committee.

2019 RESEARCH AWARDS

CONTINUATIONS

Principal Investigator	Project Title	2019 Award
Reesink, Heidi	Intra-Articular Recombinant Lubricin to restore Joint Lubrication & Prevent Osteoarthritis in Horses	$88,254
Van de Walle, Gerlinde	The Mesenchymal Stem Cell Secretome against Equine Herpesvirus Type-1 Infections	$74,578
Wagner, Bettina	Towards a Neonatal Vaccine against Equine Herpesvirus Type 1 (EHV-1)	$99,923
	SUBTOTAL:	**$ 262,755**

NEW AWARDS

Principal Investigator	Project Title	2019 Award
Antczak, Douglas	Functional gene annotation in the horse	$72,954
Cheetham, Jonathan	Accelerating recovery after Laryngeal Nerve Graft in Horses	$98,385
Delco, Michelle	The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease	$57,540
Reesink, Heidi	Does Proximal Sesamoid Bone Mineral Loss Lead to Increased Fracture Risk?	$61,351
Wagner, Bettina	Intranasal biomarkers of EHV-1 susceptibility and protection	$72,568
	SUBTOTAL:	**$362,798**

PROGRESS IN 2019

PI	Project Title	Term Date	Report Type in Appendix B
Delco, Michelle	The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease	12/31/20 with NCE*	Progress report attached
Divers, Thomas	Characterizing Tropism and Transmission of Equine Parvovirus-Hepatitis (EQPV-H)	12/31/19	Final report attached
Ducharme, Norm	Two-day tie-back (injection laryngoplasty): proof of principle	12/30/19	Final report attached
Fubini, Susan	The Relationship between Obesity and Post-Operative Incisional Infections Following Abdominal Surgery in the Horse	6/30/20 with NCE*	Progress report attached
Johnson, Philippa	Equine brain white matter: A comparative tractography and gross dissection study	11/30/19	Final report attached
Mohammed, Hussni	Factors predispose to musculoskeletal injuries and catastrophic events in racing horses	12/31/19	Final report attached
Nixon, Alan	Next Generation Arthritis Control through Lubricin and IL-1 Receptor Antagonist Overexpression in Carpal OA	12/31/19	Final report unavailable
Perkins, Gillian	Validation of an Equine Stall-side Major Crossmatch Test	5/31/2020 with NCE*	Progress report attached/ Final report pending
Reesink, Heidi	Intra-articular recombinant lubricin to restore joint lubrication and prevent osteoarthritis in horses	12/31/19	Final report attached
Reesink, Heidi	Proximal sesamoid bone microdamage and fracture toughness in Thoroughbred racehorses	5/31/20 with NCE*	Progress report attached
Van de Walle, Gerlinde	The Mesenchymal Stem Cell Secretome Against Equine Herpesvirus Type I Infections	12/31/20 with NCE*	Progress report attached
Wagner, Bettina	Intranasal Biomarkers of EHV-1 Susceptibility and Protection	12/31/20 with NCE*	Progress report attached
Wagner, Bettina	Towards a neonatal vaccine against equine herpesvirus type 1 (EHV-1)	6/30/20	Progress report attached

*NCE = No Cost Extension

EXTERNAL FUNDING

The Incentive Program enables the Fund to leverage its investment in Zweig-sponsored research by encouraging Veterinary College faculty to seek either additional or supplementary monies from external sponsors that base their award decisions on a process that involves informed scientific review. The external grant must be closely related to a Zweig project. Eligible sponsors include, but are not limited to, the Grayson Foundation, the NIH, the NSF, and the USDA's National Research Initiative. Recipients provide an annual report on the use of these funds.

No incentive awards were given out in 2019.

PUBLICATIONS

Banfield J, Lisak R, Omar A, Domingos W, Fiaschitello A, Morales-Gomez A, Divers TJ, **Mohammed HO**. Investigating the Risk of Equine Motor Neuron Disease in a Brazilian Stable and Successful Intervention. J Equine Vet Sci. 2019 Jun;77:132-138. doi: 10.1016/j.jevs.2019.02.024. Epub 2019 Mar 27. PubMed PMID: 31133307.

Cresswell EN, McDonough SP, Palmer SE, Hernandez CJ, **Reesink HL**. Can quantitative computed tomography detect bone morphological changes associated with catastrophic proximal sesamoid bone fracture in Thoroughbred racehorses? Equine Vet J. 2019 Jan;51(1):123-130. doi: 10.1111/evj.12965. Epub 2018 Jun 1. PubMed PMID: 29758110.

Donnelly CG, Sones JL, Dockweiler JC, Norberg LA, Norberg LE, Cheong SH, **Gilbert RO**. Effects of flunixin meglumine on postponement of ovulation in mares. Am J Vet Res. 2019 Mar;80(3):306-310. doi: 10.2460/ajvr.80.3.306. PubMed PMID: 30801209.

Holmes CM, Violette N, Miller D, Wagner B, Svansson V, **Antczak DF**. MHC haplotype diversity in Icelandic horses determined by polymorphic microsatellites. Genes Immun. 2019 Nov;20(8):660-670. doi: 10.1038/s41435-019-0075-y. Epub 2019 May 9. PubMed PMID: 31068686.

Miller JE, Mann S, Fettelschoss-Gabriel A, **Wagner B**. Comparison of three clinical scoring systems for Culicoides hypersensitivity in a herd of Icelandic horses. Vet Dermatol. 2019 Dec;30(6):536-e163. doi: 10.1111/vde.12784. Epub 2019 Aug 22. PubMed PMID: 31441172.

Morales Gómez AM, Zhu S, Palmer S, Olsen E, Ness SL, Divers TJ, Bischoff K, **Mohammed HO**. Analysis of neurofilament concentration in healthy adult horses and utility in the diagnosis of equine protozoal myeloencephalitis and equine motor neuron disease. Res Vet Sci. 2019 Aug;125:1-6. doi: 10.1016/j.rvsc.2019.04.018. Epub 2019 May 11. PubMed PMID: 31103855.

Perkins G, Babasyan S, Stout AE, Freer H, Rollins A, Wimer CL, Wagner B. Intranasal IgG4/7 antibody responses protect horses against equid herpesvirus-1 (EHV-1) infection including nasal virus shedding and cell-associated viremia. Virology. 2019 May;531:219-232. doi: 10.1016/j.virol.2019.03.014. Epub 2019 Mar 22. PubMed PMID: 30928700.

Schnabel CL, Babasyan S, Freer H, **Wagner B**. CXCL10 production in equine monocytes is stimulated by interferon-gamma. Vet Immunol Immunopathol. 2019 Jan;207:25-30. doi: 10.1016/j.vetimm.2018.11.016. Epub 2018 Nov 24. PubMed PMID: 30593347.

Schnabel CL, Babasyan S, Rollins A, Freer H, Wimer CL, Perkins GA, Raza F, Osterrieder N, **Wagner B**. An Equine Herpesvirus Type 1 (EHV-1) Ab4 Open Reading Frame 2 Deletion Mutant Provides Immunity and Protection from EHV-1 Infection and Disease. J Virol. 2019 Nov 15;93(22). doi: 10.1128/JVI.01011-19. Print 2019 Nov 15. PubMed PMID: 31462575; PubMed Central PMCID: PMC6819910.

Tomlinson JE, Kapoor A, Kumar A, Tennant BC, Laverack MA, Beard L, Delph K, Davis E, Schott Ii H, Lascola K, Holbrook TC, Johnson P, Taylor SD, McKenzie E, Carter-Arnold J, Setlakwe E, Fultz L, Brakenhoff J, Ruby R, Trivedi S, Van de Walle GR, Renshaw RW, Dubovi EJ, **Divers TJ**. Viral testing of 18 consecutive cases of equine serum hepatitis: A prospective study (2014-2018). J Vet Intern Med. 2019 Jan;33(1):251-257. doi: 10.1111/jvim.15368. Epub 2018 Dec 5. PubMed PMID: 30520162; PubMed Central PMCID: PMC6335536.

PATENTS

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Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2016. Novel Immunogenic proteins of Leptospira. US Patent 9,366,671, filed October 6, 2015, and issued June 14, 2016.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2015. Novel Immunogenic proteins of Leptospira. Canada Patent 2820949, filed July 26, 2013, and issued December 8, 2015.

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Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2015. Novel Immunogenic proteins of Leptospira. United Kingdom Patent EP2447278, filed August 22, 2011, and issued April 8, 2015.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2015. Novel Immunogenic proteins of Leptospira. Germany Patent 603 47 502.7, filed August 22, 2011, and issued April 8, 2015.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2015. Novel Immunogenic proteins of Leptospira. France Patent EP2447278, filed August 22, 2011, and issued April 8, 2015.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2015. Novel Immunogenic proteins of Leptospira. Europe Patent 2447278, filed August 22, 2011, and issued April 8, 2015.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2014. Novel Immunogenic proteins of Leptospira. US Patent 8,900,825, filed April 30, 2012, and issued December 2, 2014.

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Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2014. Novel Immunogenic proteins of Leptospira. Germany Patent 60345629.4, filed April 10, 2005, and issued January 15, 2014.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2014. Novel Immunogenic proteins of Leptospira. France Patent 1565080, filed April 10, 2005, and issued January 15, 2014.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2014. Novel Immunogenic proteins of Leptospira. Europe Patent 1565080, filed April 10, 2005, and issued January 15, 2014.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2013. Novel Immunogenic proteins of Leptospira. Canada Patent 2501939, filed April 10, 2005, and issued October 8, 2013.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2013. Novel Immunogenic proteins of Leptospira. Australia Patent 2010200927, filed March 10, 2010, and issued March 4, 2013.

Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2012. Immunogenic proteins of Leptospira. US Patent 8,168,207, filed October 28, 2008, and issued May 1, 2012.

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Chang, Yung-Fu, and Raghavan U. M. Palaniappan. 2010. Immunogenic proteins of Leptospira. US Patent 7,655,427, filed April 8, 2005, and issued February 2, 2010.

Heidi L. Reesink, VMD, PhD, DACVS-LA

Harry M. Zweig Assistant Professor in Equine Health 2019-2021

Heidi Reesink has been named the Harry M. Zweig Assistant Professor in Equine Health in honor of her ambitious research program to detect horses at risk for catastrophic injuries and to develop new treatments for arthritis.

The professorship is a three-year, endowed position for a junior faculty member who shows great promise for advancing equine research. It can be instrumental in helping junior faculty secure funding and develop high-level publications necessary for long-term success. Reesink has received grants previously from the Zweig Memorial Fund to support individual research projects. She has also received support from the Grayson-Jockey Club Research Foundation, the Cornell Center for Advanced Technology, the Cornell Center for Materials Research and the National Institutes of Health through a Mentored Clinical Scientist Development Award, a highly competitive grant to advance the careers of promising researchers.

"Dr. Reesink is recognized as a rising star among junior faculty and an important contributor to our college community," says Robert Weiss, Associate Dean for Research and Graduate Education. "One of the main concerns of the Harry M. Zweig Memorial Fund is catastrophic racehorse injury. Solving this problem is a critical need in the racing industry and she's doing some exciting work in that area."

Catastrophic musculoskeletal injuries – mainly broken legs – are the main cause of death for racehorses. "We would like to understand how these fractures occur and to develop better methods to screen for racehorses at risk of fracture," says Reesink.

She is working with Dr. Scott Palmer, the equine medical director for the New York State Gaming Commission, in addition to epidemiologists and pathologists to examine horses that died after sustaining fractures to their proximal sesamoid bones (PSBs) – two knobby, triangular bones at the back of the fetlock joint. Often, racehorses with this injury have no telltale signs of lameness during pre-race examinations or X-rays. Reesink is comparing the PSBs from the uninjured leg of those horses to PSBs from horses that died of other causes, using advanced CT scans. She hopes to develop better screening procedures to identify horses susceptible to fractures.

Reesink is also looking into new treatments and early detection methods for arthritis. "Joint disease and osteoarthritis are the leading cause of lameness in horses, but there are limited options for treating arthritis in horses and in humans," says Reesink. "A long term goal is to develop better therapies, that will both provide longer and better pain relief and that, ideally, will prevent or delay the development of arthritis."

After an injury, many – but not all – horses develop arthritis, so Reesink is examining the synovial fluid that bathes the joints to identify biomarkers that would indicate which horses are at risk and might benefit from preventive therapies. She is also investigating lubricin, a sugar-coated protein in the synovial fluid that provides lubrication, to see if an injection of lubricin can treat lameness. Additionally, along with pharmaceutical industry colleagues, Reesink is testing whether horses benefit from human arthritis medicines that are not available on the veterinary market.

Reesink is passionate about "one medicine," the concept that human and veterinary biomedical research can each inform the other. She believes that while providing treatment for animals, she can also offer insights to advance human health, and hopes her work will translate into clinical applications that benefit both horses and people.

As a former athlete herself, Reesink has fractured bones and injured joints while playing volleyball, competing in tae kwon do events, snowboarding and running, and so she understands the challenges and potential for developing better treatments for sports injuries. "I saw equine orthopedic surgery as a way to combine my love of the horse as well as my desire to advance the science of sports medicine."

CORNELL CLINICAL FELLOW IN EQUINE HEALTH

At the 2007 annual meeting, the Harry M. Zweig Committee approved the allocation of funds to help support a Cornell Clinical Fellow in Equine Health. Dr. Sophy Jesty was selected as Cornell's first Clinical Fellow, followed by Dr. Sarah Pownder, and more recently Dr. Joy Thomlinson. Supported in part by Zweig funds, all have been highly successful. Cornell's College of Veterinary Medicine's two-year Clinical Fellows Program is the first in the country to address a growing shortage of academic veterinarians who conduct research on animal diseases and basic biology.

The program is designed to help trainees meet the financial and time demands of qualifying for a position in veterinary academic medicine, which has traditionally required students to complete an M.S. or Ph.D. after they finish their doctorate in veterinary medicine (DVM). The two-year program, available to veterinarians who have completed a three-year residency, offers an annual salary of $65,000 plus benefits and an additional $15,000 per year to fund a research project.

There was no Clinical Fellow appointed for 2019.

APPENDIX A
Lay Summaries for New Awards

Antczak, Douglas	Functional gene annotation in the horse
Cheetham, Jonathan	Accelerating recovery after Laryngeal Nerve Graft in Horses
Delco, Michelle	The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease
Reesink, Heidi	Does Proximal Sesamoid Bone Mineral Loss Lead to Increased Fracture Risk?
Wagner, Bettina	Intranasal biomarkers of EHV-1 susceptibility and protection

Principal Investigator:	Dr. Douglas F. Antczak
Title:	Functional gene annotation in the horse
Project Period:	1/1/19-12/31/20

LAY SUMMARY

Background and significance: This renewal application seeks continued support for Cornell Veterinary College participation in an exciting and important international collaborative research effort by scientists of the Horse Genome Project. The project, called FAANG, for Functional Annotation of Animal Genomes, is defining the so-called regulatory elements in the equine genome sequence (see Diagram and Glossary below) to provide crucial new data and tools for a wide variety of applications in equine research.

Previous grants from the Zweig Fund enabled the Antczak laboratory to play a major role in the global collaboration that produced the equine genome sequence that is now freely available via the Internet to researchers worldwide in several databases. Knowledge of the genome sequence facilitated the development of new assays such as the equine Single Nucleotide Polymorphism (SNP) chip and platforms for measuring gene expression. The availability of the horse genome sequence and associated genome-scale assays have profoundly influenced equine research worldwide in many disciplines, including classical genetics, immunology and infectious diseases, stem cell biology, cartilage biology and joint disease, and allergies and autoimmunity. Cornell's contribution included the development of microsatellites for the equine linkage map, the Bacterial Artificial Chromosome (BAC) library for physical mapping and Fluorescent In Situ Hybridization (FISH), and the development and breeding of the unique lines of Major Histocompatibility Complex homozygous horses. One of those horses, the Thoroughbred mare Twilight, was selected as the DNA donor for the equine genome sequence that was produced with over $20 million in funding provided by the NIH Genome Research Institute.

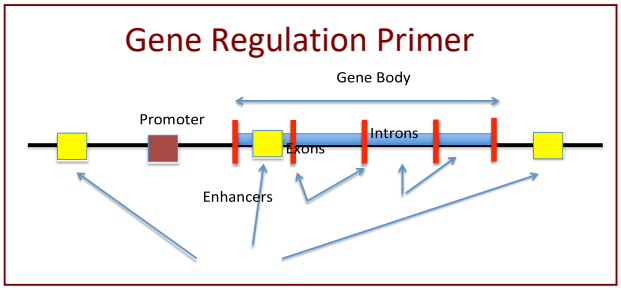
Here at Cornell virtually every equine research project now uses information from the Horse Genome Project in one form or another. As examples, in collaboration with Dr. Samantha Brooks of the Animal Science Department at Cornell, the Antczak lab used the first version of the equine SNP chip to discover the mutation causing Lavender Foal Syndrome in the Arabian breed and to develop a simple molecular test for carrier detection that is now available to breeders commercially. More recently, we identified genetic determinants influencing equine sarcoid, the most common tumor of the horse and a complex cancer with a viral origin. The Ainsworth, Felippe, Fortier, Nixon, Reesink, van de Walle, and Wagner labs have also all used information from the Horse Genome Sequence in their research.

Despite this progress, much remains to be discovered about how the information encoded in the genome is translated into healthy horses, and how it is disrupted in disease. Although the protein coding genes of the horse are now quite well defined, these represent only about 3% of the genome sequence. The remaining 97% contains the so-called non-coding parts of the genome. These are the stretches of DNA sequence that lie between and among the genes. In these stretches of DNA are areas that function as molecular switches to control whether a nearby gene is activated (switched on) or switched off (see diagram below). The non-coding elements of the equine genome that control gene transcription and other aspects of gene expression remain largely uncharacterized and are poorly understood. This is a major knowledge gap. The goal of our project is to identify these molecular switches. This is called functional genome annotation. Such information is crucial to exploiting the full potential of genomics to advance equine medicine. Evidence from parallel studies of human genetic disease and physiology has indicated that genetic change in the areas of these molecular switches has a major influence on complex diseases and physiological traits.

Progress Report: Under our current Zweig grant we are collaborating with Dr. Charles Danko of the Baker Institute to apply new molecular assays and computational programs developed in part by the

Danko lab that can speed progress in functional annotation of the equine genome. Over the past 18 months we have generated important new data from equine placenta, immune system cells called lymphocytes, and liver. We are currently analyzing our data and comparing our results to those produced by other members of the equine FAANG working group, who are using older, more laborious and expensive methods to obtain equivalent data. We are confident that the new methods developed at Cornell will produce high quality genome annotation data more efficiently and at lower cost than using the current existing assays. If we are correct, this will enable Cornell to make a major contribution to this exciting second phase of the Horse Genome Project.

Experimental Plans: In the coming two years we propose to continue our current efforts in functional annotation of the equine genome. In **Aim 1** we will determine the molecular pathways that govern development of the horse placenta, with special emphasis on the early stage of gestation between days 30 and 40, when many pregnancies are lost for unknown reasons. In **Aim 2** we will compare gene regulation among different subsets of horse lymphocytes. This will contribute to our understanding of the horse immune system and thus has relevance to applications in equine vaccine development. In **Aim 3** we will compare our results obtained with equine liver samples with data from the same samples obtained by other members of the equine FAANG consortium. This will provide a formal test of the utility of the Cornell approach to functional genome annotation. Based on our current data we are confident that the new Cornell methods will make a major contribution to the efforts of the Horse Genome Project in the FAANG consortium.



***Schematic diagram** of the region surrounding a single mammalian gene, showing DNA regions that contain Enhancer elements (yellow), the main Promoter region (purple), the Exons that encode the protein sequence (red bars), and the Intron areas that lie between Exons (thick blue line).*

Typically a gene has only a single Promoter region, but multiple Enhancers, which can be located before, after, or within genes, and sometimes long distances away from the gene body. The goal of this project is to identify the Promoter and Enhancer regions of the horse genome and the Transcription Factor proteins that bind to those regions to activate gene expression.

Glossary and Definition of Terms

DNA	Deoxyribonucleic Acid – the building block of the genome sequence
FAANG	Functional Annotation of Animal Genomes
Transcription	The process by which the information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA), eventually leading to protein production.
Transcription Factor	Proteins that bind to Enhancer and Promoter sites to activate gene expression
Promoter	Regulatory region of DNA that initiates transcription of a particular gene. Promoters are usually found just before the start of the coding gene
Enhancer	Regulatory region of DNA that can be bound by transcription factors to enhance expression of an associated gene
Introns	Areas of nucleotide sequence within a gene that are removed by during the process of protein production. Introns are part of the non-coding regions of the genome.
Exons	The parts of a gene that encode the protein sequence. The sum of all exons makes up the ~3% of the genome termed the coding sequence.

Gene Body	The length of DNA that includes the Exons and Introns of a single gene.

Deliverables and anticipated clinical application: Data generated by this Cornell genomics project will be made available to the Horse Genome Project laboratories participating in the FAANG initiative prior to publication through the periodic Horse Genome Project workshop meetings. We anticipate that knowledge of regulatory elements in the horse will be applied to many types of equine disease conditions and to equine sports performance physiology.

Future Direction: We will apply to external grant-giving bodies (NIH, Morris Animal Foundation, etc.) to extend and expand our work in functional annotation of the horse genome.

Trainee participation: It is anticipated that some Cornell undergraduate pre-veterinary students and veterinary students awarded Havemeyer Foundation Summer Research Fellowships will participate in this project.

Principal Investigator:	Dr. Jonathan Cheetham
Title:	Accelerating recovery after Laryngeal Nerve Graft in Horses
Project Period:	1/1/19-12/31/20

LAY SUMMARY

Recurrent laryngeal neuropathy (RLN) or "Roaring" is a major cause of poor athletic performance affecting 8% of racehorses and a higher percentage of sport horses. The disease affects the ability of the nerve to conduct a signal from the brain to the muscle that opens the larynx (voice box) at exercise -the *cricoarytenoid dorsalis* (CAD) muscle. In affected horses the impulses carrying this signal down the nerve travel more slowly and do not reach the muscle as effectively as in normal horses. This leads to a reduction in the size and strength of the CAD muscle causing collapse of the larynx at exercise with consequent reduced airflow and abnormal noise production.

Current standard of care for RLN is the placement of a fixed and permanent laryngoplasty suture (a "tie-back") to keep the larynx open. While this method is relatively successful in the treatment of airway obstruction in RLN affected horses, it does not restore function to the airway and can be associated with risks such as coughing and failure of the suture to hold the airway open. At the Equine Performance Clinic, we see a large number of cases each year that show early signs of RLN but are not yet sufficiently affected to warrant the cost or potential risks of this surgery. Here, we propose a regenerative approach to restore normal laryngeal function in horses affected by RLN using an enhanced nerve graft. The approach would avoid interfering with the normal protective mechanisms of the airway and so also avoid the complications associated with the current treatment. Previous attempts to restore muscle function used nerve-muscle pedicle grafting to bring a new nerve supply to the affected CAD muscle. This technique only reinnervates a small portion of the muscle. In this proposal, we use a technique which allows us to reinnervate the entire CAD muscle, accelerate reinnervation and promote recovery.

We have recently used a similar approach to restore laryngeal function in dogs. Following acute recurrent laryngeal nerve transection and graft with the phrenic nerve, spontaneous arytenoid abduction was visible within 7 weeks of surgery.

Over the last three years and with support from the Harry M. Zweig Memorial Fund and the National Institutes of Health we have begun to understand the basic mechanisms behind the role of a particular type of immune cell – the macrophage - in peripheral nerve repair. These cells are the major cell type migrating to the repair site and are the 'conductors of the orchestra', laying down tiny capillary networks along which other cell types can migrate. We have developed a sophisticated technique to isolate macrophages from the site of peripheral nerve injury. Using this technique, we evaluated how genes expressed by these macrophages change over time after injury and how genes that control the types of macrophages at the injury site affect repair after nerve graft. We have also shown that these cells change when there is a delay between injury and nerve graft, leading to modification of the microenvironment at the injury site and decreased recovery. We have also shown that these effects can be reversed using a small molecule that reduces inflammation, and that this reversal leads to improved recovery.

The overall goal of the experiments in this proposal is to change the type of macrophages at the site of nerve graft using a stable hydrogel that supports nerve growth and allows us to add small molecules called cytokines that can alter the type of macrophages. This system is safe and biocompatible. We anticipate that this approach will ameliorate the nerve degeneration and muscle atrophy, commonly observed in RLN, and restore full function. We will perform our experiments using the horse and mimicking the clinical situation of recurrent laryngeal neuropathy (RLN).
We hypothesize that by manipulating the microenvironment at the site of nerve graft and changing the function of macrophages, this will allow re-growing nerve axons to cross the repair site more rapidly

and functional recovery will be faster and better. Our preliminary data already show a very positive effect.

Our experimental approach closely mimics the situation in equine RLN where the distal nerve stump (close to the muscle) is denervated through axonal loss and demyelination and could be reinnervated by a graft using the first cervical nerve. The first cervical nerve is active during inhalation and so is an ideal candidate for grafting to the recurrent laryngeal nerve. The graft would be performed immediately behind the larynx so, if axons cross the repair site rapidly, we could anticipate reinnervation and functional recovery at the larynx to occur within 7-16 weeks. Our preliminary data from dogs support this. This approach would be a major improvement in currently available treatment options for this challenging condition.

Principal Investigator:	Dr. Michelle L. Delco
Title:	The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease
Project Period:	1/1/19-12/31/20

LAY SUMMARY

Background: There is arguably no more urgent health issue to the short- and long-term welfare of equine athletes, and therefore the future of the horseracing industry, than that of traumatic joint injury. Cartilage provides near-frictionless joint surfaces and cushioning to protect underlying bone. Evidence suggests that even mild cartilage damage can impair its ability to dissipate loads, exposing the underlying bone to repeated micro-trauma, which can ultimately lead to fracture and break-down injury. Therefore, understanding how cartilage responds to mechanical forces and perpetuates damage signals throughout the joint is critical to preventing joint trauma in equine athletes. Recent work by our group revealed that mitochondrial dysfunction is one of the very earliest responses of cartilage to overloading. Mitochondria are best known as the "powerhouses" of cells, because these organelles produce the energy required for normal tissue function and repair. Remarkably, mitochondria also act as mechanotransducers; they sense physical forces applied to tissue and convert those signals into biological responses. In other tissues, injury-induced mitochondrial dysfunction causes cells to release danger signals, or mDAMPs (mitochondrial Damage Associated Molecular Patterns). These mDAMPs can act as molecular triggers, inducing inflammation and perpetuating tissue damage. However, the role of mDAMPs has not been investigated in association with joint trauma in horses, or in other species.

Hypothesis: mDAMPs are released from chondrocytes in response to injury-induced mitochondrial dysfunction. Furthermore, mDAMPs in equine synovial fluid are associated with 1) cartilage and bone changes after experimental cartilage injury, and 2) clinical signs after naturally-occurring joint injury.

Specific Aims: Broadly, **Aim 1** will investigate the types of injury that lead to extracellular mDAMP release by cartilage. More specifically, **Aim 1a** will test the hypothesis that mitochondrial dysfunction is a specific trigger for mDAMP release**.** Chondrocytes grown in culture will be stressed with several compounds, including a general inflammatory stimulus (IL-1β), an oxidant (hydrogen peroxide), and three specific inducers of mitochondrial dysfunction (oligomycin, rotenone, FCCP). Culture media will be analyzed for three mDAMPs: 1) Mitochondrial DNA (mtDNA) will be measured by quantitative PCR, 2) The mitochondrial protein cytochrome C will be quantified by ELISA, and 3) the mitochondria-specific phospholipid cardiolipin will be quantified by TTAPE-Me assay. **Aim 1b** will investigate if mDAMP release from cartilage occurs after mechanical overloading. Previous work by our group revealed that mitochondrial dysfunction is an immediate response of cartilage to impact-injury. In that study, live cartilage explants were injured, then maintained in culture for one week and media was harvested and frozen. This banked media will be analyzed for mDAMPs, as described above. *Results of Aim 1 will provide insight into how mDAMP release is triggered, and therefore how it may be manipulated therapeutically.*

Specific **Aim 2** will analyze mDAMPs in equine joint fluid, in order to determine if mDAMPs are a useful indicator of early cartilage/bone injury. Joint fluid previously collected from 1) horses that have had experimental injury to their articular cartilage, and 2) horses with naturally occurring joint injuries will be analyzed as described in Aim 1. The concentration of mDAMPs will be compared to microscopic changes in cartilage and bone after experimentally induced joint injury, and to clinical findings (lameness, joint swelling, radiographic bone sclerosis, cartilage damage at arthroscopy, etc.) in clinical patients presenting with joint injury. Three sources for clinical samples will be utilized; cases presenting to the Cornell Hospital for Animals in Ithaca, NY, cases presenting to Cornell Ruffian Equine Specialists in Elmont, NY and historic samples already available through the Cornell Biobank.

Results of Aim 2 will determine if mDAMPs are a useful indicator of sub-clinical joint injury in horses, and which horses may benefit from early intervention.

Building on previous work and utilizing samples (in Aims 1b and 2) that have been previously harvested and banked will allow us to complete the proposed studies, while obtaining the maximum amount of information within the 2-year time frame of this proposal.

Relevance to equine health and racing: Understanding the role of mDAMPs has the near-term potential to change the way we diagnose and treat joint injury in horses. Currently available therapies only mask pain, and are instituted after irreversible cartilage and bone damage have already occurred, predispoing horses to further injury. The goal of this research is to identify early/subclinical joint injury, and to develop targeted disease-modifying therapies to break the cycle of ongoing damage. For example, several drugs are being investigated which could block mDAMP release, and act as anti-inflammatories while protecting cartilage and underlying bone. mDAMPs are promising candidate biomarkers, and screening in joint fluid could serve as a practical test for early joint damage, to identify horses requiring therapy or modified training programs. This grant will provide data in support of a larger NIH grant proposal (R01) to further investigate these questions.

Principal Investigator:	Dr. Heidi Reesink
Title:	Does Proximal Sesamoid Bone Mineral Loss Lead to Increased Fracture Risk?
Project Period:	1/1/19-12/31/20

LAY SUMMARY

The Research Problem

Catastrophic breakdown injuries are of utmost concern to the racing industry. Aside from the unfortunate loss of equine life and risk to jockeys, racehorse fatalities negatively impact public perception and lead to substantial economic losses for the equine industry. Racehorses are predominantly affected by injuries to the fetlock (ankle) joint, with fractures of the proximal sesamoid bones (PSBs) most over-represented in causes of racehorse fatality in New York, California, Kentucky, Florida and Hong Kong. Proximal sesamoid bone (PSB) fracture was the most common fatal musculoskeletal injury in horses on New York racetracks from 2013-2015, accounting for 35.3% of fatalities[1] **(Fig 1)**.

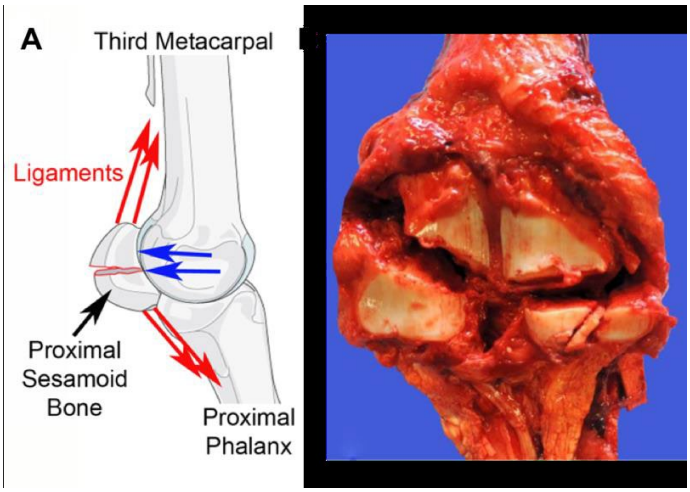


Fig 1. A) *A diagram displaying the tensile forces from ligaments and compressive forces from the articulating cannon bone (MC3) condyles on the PSBs.* ***B)*** *Biaxial PSB fracture with severe comminution of the base of the medial PSB. The presence of a sagittal fracture line dividing the base of the medial PSB is a common finding in NY racehorses. Note the presence of score lines in the articular cartilage of the PSBs, corresponding to evidence of cartilage injury. Figure adapted from Palmer et al. 2017[1].*

However, the underlying causes or bone changes leading to catastrophic PSB fracture are poorly understood, including whether or not PSB fracture is associated with pre-existing osteoarthritis (OA) or changes in bone mineral density and quality. While some imaging studies suggest that horses have more advanced arthritic changes in the opposite forelimb[2,3], recent data from a small pilot study in our lab suggests that horses that sustain catastrophic PSB fracture have more evidence of OA than control racehorses and more advanced OA in the fractured limb as compared to the intact limb **(Preliminary Studies, Fig 2)**. However, limitations of this pilot study are the small sample size (n=16 horses total; n=8 PSB fracture, n=8 racehorse controls) and broad age range (3-10 yo.) of horses included. Horses ranged from having career durations from 3 to 415 weeks, or 0 to 59 races, making conclusions about pre-existing osteoarthritis and bone shape/predisposing factors confounded by extreme variation in career durations and exercise histories[4]. For example, although there appeared to be differences between racehorse fractures and racehorse controls with respect to OA, histologic OA changes were also significantly impacted by career duration ($p<0.0001$) and total furlongs ($p<0.0001$).

There are currently no tests or imaging techniques that can be used to predict which horses are at increased risk of catastrophic PSB fracture, and horses undergoing catastrophic fracture rarely have localizing signs (e.g., lameness, joint effusion, heat) prior to the injury. In our pilot study in New York racehorses, catastrophic PSB fracture was NOT simply due to too much exercise or too many accumulated furlongs, as both unraced and 2-year-old horses sustained catastrophic PSB fracture[4]. In addition, horses that sustained catastrophic PSB fracture exercised LESS per week than controls **(Experimental Approach, Fig 6)**. While it is possible that different mechanisms are responsible for

PSB fracture in young, unraced horses and older horses with more total furlongs accumulated, researchers cannot answer these questions without being able to examine a sufficient number of fetlock joints and PSBs from both young (2-3 yo.) and old(er) horses.

In addition to the finding that horses that experienced catastrophic PSB fractures exercised LESS per week than controls, we also found that horses with PSB fractures had increased bone volume fraction, or the proportion of bone occupied by mineralized tissue vs. unmineralized tissue. However, somewhat counterintuitively, bone from horses with PSB fractures had *increased* bone volume fraction but *decreased* bone mineral density. The difference between bone volume fraction and bone mineral density are explained in more detail in Specific Aim 2 in the Experimental Approach (see water glass volume analogy in **Fig 5**), but our pilot study was the first study to report that there is less mineral in bone from horses sustaining PSB fractures as compared to controls. This may be because our study was the first to perform bone mineral density 5 measurements using the gold-standard measurement of ash fraction, or heating of the bone to 600°C, to measure the amount of mineral. This is a very interesting finding because bone mineral content, or bone mineral density, are important measures of bone quality and bone strength. In humans, bone mineral density is the most important quantitative measurement used to predict fracture risk. Thus, although equine PSBs have very high bone volume fractions as compared to humans (e.g., ~ 87% vs. 22%), it is possible that insufficient mineral, or *decreased* bone mineral density, may lead to fracture risk in horses, too. Therefore, the goal of this study is to measure bone mineral density in a larger population of horses, including non-racehorse controls, and to compare various non- or minimally-invasive measurements of bone mineral density to the gold-standard measurement of ash fraction. Our goal is to validate the preliminary findings that we have observed with respect to bone volume fraction and OA in the pilot study, and to add quantitative measurements of bone mineral density to epidemiological models to improve accuracy of fracture risk prediction in Thoroughbred racehorses.

The Long-Term Goal—Non- or Minimally-invasive Prediction of Fracture Risk: Quantitative bone shape and quality parameters, including bone volume fraction and/or bone mineral density, can be measured using computed tomography (CT, or CAT scan), dual x-ray absorptiometry (DXA, or bone density scan) and Raman spectroscopy (a light scattering technique that provides a “chemical fingerprint” for identifying molecules in a tissue). These modalities are rapidly being adapted for use in the standing horse, with several companies or start-ups actively pursuing standing CT (i.e., Mobius Imaging, LLC; Limited View 3D Imaging, Kawcak) using distinct technologies. DXA mobile units are available and routinely used in human medicine to screen patients at risk of osteoporotic fracture. A DXA unit was recently tested and validated for use in the equine distal cannon bone[5], demonstrating potential applicability of this modality in the standing horse. Finally, Raman spectroscopy is currently in pre-clinical testing for *in vivo* use[6], so all of these “research” modalities will likely have clinical applicability within the next few years. However, before gaining widespread clinical use, we first must determine which parameters are most highly correlated with catastrophic PSB fracture and which modalities best compare to the gold-standard bone mineral density measurements obtained by ashing bone. These side-by-side measurements can only be performed in cadaver tissue; therefore, there is a critical need to determine which parameters are useful and which technologies should be pursued *in vivo* in the training and racing Thoroughbred.

The human FRAX® fracture risk prediction tool takes advantage of many patient variables, including age, sex, family history, prior history of fracture, medication use (e.g., glucocorticoids = steroids) and bone mineral density measurements. Although the parameters that we include in the racehorse model will likely differ in some respects to the FRAX® model, the premise of combining intrinsic factors, such as sex and age, with exercise data and quantitative bone quality information is likely to result in the most useful predictive model. Similar to the FRAX® model, there are several steps that must first be completed. The first step is to identify the most useful quantitative parameters to include in the model

and to determine which imaging modalities provide the most useful information, which we plan to perform in our Research Plan.

Research Plan

Our experimental approach builds upon a currently-existing CT imaging database of TB racehorse fetlocks and includes bolstering racehorse fracture and control numbers (n=5 fractures, n=5 controls for a total of 20 fractures and 20 controls) and adding a non-racehorse control population (n=20) in Aim 1. In addition, we propose to measure bone mineral density using both clinical and micro-CT, which was not performed in the pilot study due to variations in PSB storage and lack of a density phantom. In Aim 2, we propose to test several non- or minimally-invasive imaging modalities (i.e., clinical CT, micro-CT, DXA, and Raman spectroscopy) to measure PSB mineral density as compared to gold-standard destructive ash fraction measurements. We will also measure bone mineral density in the iliac crest (hip bone biopsy site) to determine how well density measurements correlate between PSBs and a remote biopsy site. Finally, in Aim 3, we will determine whether quantitative bone mineral density, bone volume fraction or OA measurements can improve the ability of epidemiological models to accurately predict PSB fracture. The long-term objective of this research is to develop non- or minimally-invasive techniques that will aid veterinarians, trainers and horse owners in identifying which racehorses are at increased risk of catastrophic proximal sesamoid bone (PSB) fracture.

Principal Investigator:	Dr. Bettina Wagner
Title:	Intranasal biomarkers of EHV-1 susceptibility and protection
Project Period:	1/1/19-12/31/20

LAY SUMMARY

Equine herpesvirus type 1 (EHV-1) frequently causes severe neurologic outbreaks of equine herpesvirus myeloencephalopathy (EHM) in horse populations (Kydd et al. 2006, Lunn et al 2009, Perkins et al 2009). The increased morbidity and mortality due to the neurologic manifestation of EHV-1 has prompted increased biosecurity (Henninger et al 2007, Kohn et al 2006, Perkins et al 2009). During EHM outbreaks, horses are typically quarantined for several weeks. The medical and economic impact of EHV-1 outbreaks is often substantial through lost training and competing time, costs related to quarantine, treatment, and loss of horses due to death of severely neurologic horses (Goehring et al 2006, Lunn et al 2009).

Currently, EHV-1 outbreaks are confirmed by PCR detecting pathogen DNA in the nasal secretion of infected horses. This is a sensitive technique but does not take into account the stage of EHV-1 infection or existing host immunity against EHV-1. Consequently, all horses on the outbreak grounds are quarantined for several weeks independent of infection stage and immune status. The existing PCR assays confirm EHV-1 infection in nasal secretion samples (typically nasopharyngeal swabs) by detecting EHV-1 DNA. In infected horses, the PCR is positive as long as viral DNA is present in the sample (Elia et al. 2006). However, viral DNA is detectable for much longer than infectious virus is shed. For example, after the onset of immunity neutralized virus is taken up by macrophages residing in the respiratory tract and is no longer infectious but will still result in a positive PCR result. Methods and markers that give additional information on the immune status of EHV-1 PCR positive horses are missing. Quarantine is consequently extensive and driven by pre-caution. A better understanding of when a PCR positive horse is still transmitting virus and can infect other horses, or when it developed immunity and viral DNA processed by immune cells is no longer a risk factor for other horses, will improve EHV-1 quarantine management and reduce costs associated with EHM outbreaks.

Our previous research funded by the Harry M. Zweig Memorial Fund for Equine Research and USDA/NIFA has shown that fully immune, protected horses are not shedding virus or developing clinical disease (Figure 1). The conclusions from our EHV-1 host immune and protection studies are: (1) a protected horse will not transmit the virus to another horse even if it was exposed to EHV-1; and (2) viremia is not happening in fully protected horses. Viremia is a pre-requisite for developing neurologic disease (Edington et al. 1986, Borchers et al. 2006, Pusterla et al. 2009). Thus, fully protected horses are at no risk of developing EHM.

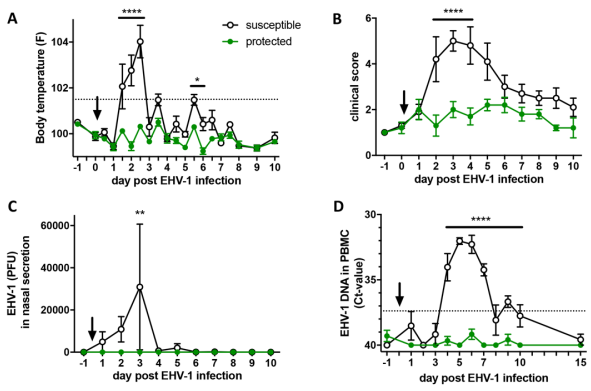


Figure 1. *Susceptible and protected horse after experimental EHV-1 infection. (A) Body temperature, (B) clinical signs, (C) nasal viral shedding and (D) viremia in EHV-1 susceptible and protected horses. Susceptible and protected horses were infected (arrow) with 1x10^7 Pfu of the neurogenic EHV-1 strain Ab4. Significant differences between the two groups are indicates by asterisks: * p<0.05, ** p<0.01, **** p<0.0001.*

These recent findings initiated the development of a novel diagnostic assay for EHV-1 host immunity that was designed to distinguish protected horses from those that are susceptible. The latter group will develop disease and spread the infection, the former will not. The assay also supports the identification of the infection stage in horses that are susceptible and get clinically ill during an EHV-1 outbreak

(Figure 2). The biomarker identification in susceptible horses during the course of infection resulted from projects funded by the Zweig Fund and experimental EHV-1 infection studies at Cornell (Wagner et al. 2017, Schnabel et al. 2018).

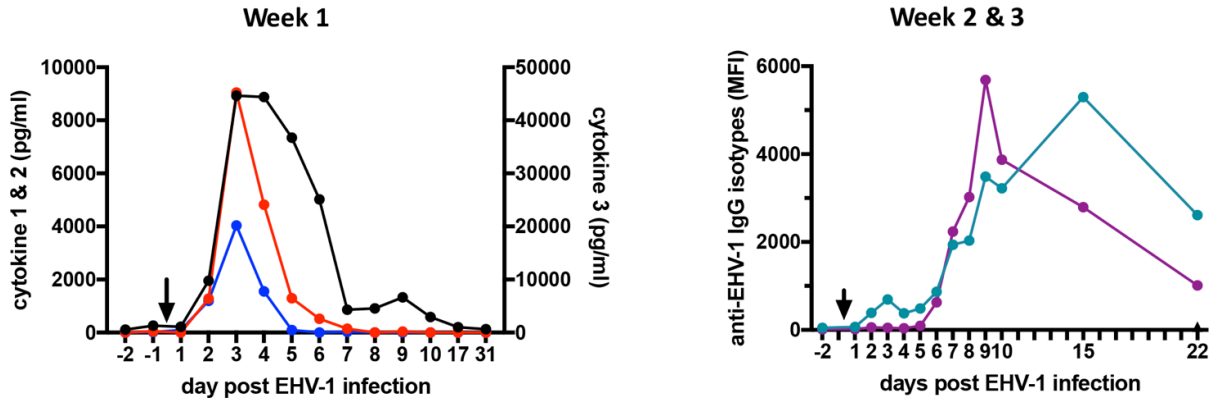


Figure 2. *Intranasal immune response and biomarkers to identify the infection stage with EHV-1. Different intranasal biomarkers increase and decline at different times after experimental infection with 1x10^7 Pfu EHV-1 in the nasal secretion. The biomarker changes are used in the novel EHV-1 Immune Biomarker assay to support the management of EHV-1 outbreaks in the US.* ***Left panel****: During week 1 three cytokine markers are detectable in nasal secretions of infected, susceptible horses. The cytokine biomarkers peak on day 3 post infection (pi), decline afterwards and become undetectable again by day 5-7 pi, with the exception of cytokine 3 that is maintained at low levels for another week.* ***Right panel****: IgG isotypes start rising in the nasal secretion in week 2. One of the IgG isotypes peaks early around day 9 pi and declines afterwards. Another IgG isotype increases more slowly, peaks at the end of week 2 pi and then slowly declines. In a clinical sample, the IgG isotype ratio of these two isotypes allows to distinguish horses that are beyond week 2 pi. These horses are immune, protected against disease induced by EHV-1, and past the infectious viral shedding phase.*

The EHV-1 Immune Biomarker assay will become available this fall at the Animal Health Diagnostic Center at Cornell University to improve the management of EHV-1 outbreaks in the US. It measures biomarkers in nasal secretion of horses that are indicators for the stage of EHV-1 infection and protective immunity. The assay can be used to identify and distinguish (i) susceptible horses (will develop disease during an outbreak) from those that are in (ii) the early infection stage (high shedders of virulent pathogen), (iii) the later infection stage (developing immunity, low or no shedding), and (iv) immune horses that will not develop disease or shed virus.

The use of the EHV-1 Immune Biomarker assay for the identification of horses in the different infection stages supports the separation of these groups in an outbreak situation, will help to reduce new infections and the overall time of the quarantine by improving management of these groups, will allow to release immune horses earlier from quarantine, and gives veterinarians and horse owners a better tool to evaluate risk and prognosis for each horse. Importantly, shorter quarantine will significantly decrease costs during EHV-1 outbreaks.

The new EHV-1 Immune Biomarker tool described above was developed for immune parameters that can currently be measured in horses at the protein level. These are limited due to the restricted availability of immune reagents and assays for horses. In this project, we propose to use banked intranasal samples from our previous EHV-1 challenge and protection studies to comprehensively analyze gene expression in EHV-1 susceptible and protected horses. For each sample, clinical, virological and immunological study outcomes are documented. These banked samples represent a well characterized, valuable set of materials to improve our understanding of the viral pathogenesis in

and transmission from the upper respiratory tract. The novel biomarkers identified during this project will be added to the new assay described above to further improve the diagnostic EHV-1 toolkit for horses and informed decision making during EHV-1 outbreaks and quarantine.

The **outcome of this project** will be the identification and evaluation of several currently unknown intranasal host protection markers during EHV-1 infection to improve our understanding of protective immunity and risk of transmission. New detection tools will be developed to result in an advanced EHV-1 Immune Biomarker assay. The findings and the new diagnostic assay tool will directly support the management of EHV-1 outbreaks and reduce medical and economic losses for the horse industry.

APPENDIX B
Final & Progress Reports from 2019

PI	Title	Report Type
Delco, Michelle	The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease	Progress
Divers, Thomas	Characterizing Tropism and Transmission of Equine Parvovirus-Hepatitis (EQPV-H)	Final
Ducharme, Norm	Two-day tie-back (injection laryngoplasty): proof of principle	Final
Fubini, Susan	The Relationship between Obesity and Post-Operative Incisional Infections Following Abdominal Surgery in the Horse	Progress
Johnson, Philippa	Equine brain white matter: A comparative tractography and gross dissection study	Final
Mohammed, Hussni	Factors predispose to musculoskeletal injuries and catastrophic events in racing horses	Final
Nixon, Alan	Next Generation Arthritis Control through Lubricin and IL-1 Receptor Antagonist Overexpression in Carpal OA	Unavailable
Perkins, Gillian	Validation of an Equine Stall-side Major Crossmatch Test	Progress/ Final pending
Reesink, Heidi	Intra-articular recombinant lubricin to restore joint lubrication and prevent osteoarthritis in horses	Final
Reesink, Heidi	Proximal sesamoid bone microdamage and fracture toughness in Thoroughbred racehorses.	Progress
Van De Walle, Gerlinde	The Mesenchymal Stem Cell Secretome Against Equine Herpesvirus Type I Infections	Progress
Wagner, Bettina	Intranasal Biomarkers of EHV-1 Susceptibility and Protection	Progress
Wagner, Bettina	Towards a neonatal vaccine against equine herpesvirus type 1 (EHV-1)	Progress

Principal Investigator:	Dr. Michelle L. Delco
Title:	The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease
Project Period:	1/1/19 – 12/31/20
Reporting Period:	1/1/19 – 12/31/19

Summary of Progress*:* Our broad aims were to investigate the types of signals that initiate the release of mitochondria-specific Damage Associated Molecular Patterns (mDAMP) by injured cartilage. We used chemicals that stress cells in different ways (causing an inflammation response or inhibiting specific mitochondrial functions). We have largely completed the evaluation of mtDNA as described in our first aim, and our studies have led to additional questions; we have added experiments to ensure optimal data normalization, and to evaluate environmental conditions that may contribute to baseline cellular stress and/or influence cell function in chondrocytes, and which appear to influence mDAMP release. Since Aim 1b requires new bovine-specific primers, we have proceeded to analyzing equine synovial fluid samples from experimental joint injury and gained exciting information for additional experiments (see data below). We have also begun to collect and bank clinical samples for future analysis.

Principal Investigator:	Dr. Thomas Divers
Title:	Characterizing Tropism and Transmission of Equine Parvovirus-Hepatitis (EQPV-H)
Project Period:	1/1/18 – 12/31/19
Reporting Period:	1/1/18 – 12/31/19

A. Specific Aims of the Study and Modifications

Aim 1: Determine EqPV-H tissue and cellular tropism. Not changed.

Aim 2: Optimize an *in vitro* cell culture system for EqPV-H. Not changed.

Aim 3: Determine EqPV-H transmission via stem cell treatment. Expanded. We were able to perform additional inoculation studies to examine viral shedding and nasal and oral transmission.

B. Summary of Scientific Findings

Aim 1: Determine EqPV-H tissue and cellular tropism. Tissues were collected from 3 horses in acute viremia and in 3 horses >15 weeks after infection. Tissues were screened by PCR for EqPV-H DNA. The highest viral load was found in the liver of acutely infected horses, although EqPV-H DNA was present at low levels in many tissues in both acutely and chronically infected horses. Liver of experimentally infected horses were examined by *in situ* hybridization (ISH), which demonstrated that EqPV-H infects hepatocytes specifically, and pre-treatment with DNase demonstrated the presence of viral RNA in hepatocytes, indicating viral replication in these cells. These experiments demonstrated that EqPV-H is hepatocytotropic.

Aim 2: Optimize an *in vitro* cell culture system for EqPV-H. Inoculation of a variety of equine cells lines (mesenchymal stromal cells from multiple sources, fibroblasts) and a human hepatoma cell line with EqPV-H in horse serum did not yield detectable infection. An equine primary hepatocyte culture system was developed for culture in monolayer and two rounds of culture and inoculation with EqPV-H in equine serum showed promising results, although the amount of virus produced was low. The cells survived a maximum of 7 days, which is suspected to be insufficient for robust viral replication, as horses infected in vivo do not become viremic for 1-4 weeks. Efforts are ongoing in our lab to optimize 3-D culture systems for equine liver via explant or organoid methods.

Aim 3: Determine EqPV-H transmission via stem cell treatment and natural horizontal transfer. Bone marrow aspirates were collected from 3 highly viremic horses. Culture of bone marrow-derived mesenchymal stromal cells (BM-MSC, aka stem cells) from these aspirates demonstrated that EqPV-H contamination of MSC products is most likely due to equine serum carryover. Cells cultured only in FBS had low to undetectable amounts of EqPV-H remaining at the time of therapeutic use, whereas cells cultured in autologous equine serum containing high viral load of EqPV-H had higher loads of EqPV-H in the final preparation for therapeutic use despite washing off the serum. In our tests, we were able to transmit EqPV-H by injection into the stifle joint (n = 2) or injured superficial digital flexor tendon (n = 3) when high viral dose was administered by injecting either straight equine serum, or MSC resuspended in equine serum. This demonstrated that EqPV-H can be taken up from these sites and generate systemic infection. However, we did not demonstrate infection after inoculation with MSC cultured in FBS (n = 1, undetectable EqPV-H) or MSC cultured in equine serum but resuspended in PBS for injection (n = 3).

Because EqPV-H is highly prevalent even in horses which have not been treated with equine biologic products, we considered that natural routes of horizontal transmission must occur. Previous funding had allowed us to test horse fly transmission, which was unsuccessful in a limited number of attempts.

Although this did not completely rule out biting fly transmission, it suggested that fly transmission was not highly efficient. Therefore, we began to screen nasal, oral, and fecal excretions of experimentally infected horses and found that EqPV-H is shed via all three routes during high viremia. Therefore, we developed the new hypothesis that EqPV-H might be transmitted by either inhalation or ingestion. This was tested by inoculating 2 horses each with EqPV-H in 1ml equine serum by intranasal spray and by mouth. One horse was successfully infected by oral inoculation, demonstrating this is a possible route of horizontal transmission.

In the process of this Aim, we had multiple horses experimentally infected with EqPV-H which were either used for the tissue tropism in Aim 1 or were followed by serial serum biochemistry and liver biopsy to observe pathogenicity of the infection. Altogether, there were 10 horses monitored for pathogenic effects and 8 of 10 demonstrated hepatitis evidenced by elevation in at least 2 liver markers. Hepatitis was characterized by lymphocytic infiltrate of the liver with individual hepatocyte necrosis, particularly in the centrilobular region. Necrotic hepatocytes were EqPV-H DNA positive by ISH. These findings are a milder version of the classic findings in Theiler's disease.

C. Significance

Emphasize the significance of the findings and their potential impact.

Equine parvovirus-hepatitis (EqPV-H) was only recently discovered and it has broad distribution in equine populations worldwide. While the connection with equine Theiler's disease (acute hepatic failure) had been demonstrated by our recent case series, proof of causation between infection and disease was not confirmed. This study was designed to evaluate the tissue and cellular tropism of EqPV-H to determine whether it could be consistent with the proposed liver pathogenicity. Additionally, we examined multiple transmission modalities to develop methods of controlling disease spread.

Our salient findings from this study were that EqPV-H is hepatocytotropic, pathogenic, and horizontally transmitted by iatrogenic and natural routes. Infection is associated with hepatitis, which is demonstrated by biochemical and histopathologic findings consistent with naturally occurring cases of Theiler's disease. EqPV-H is shed via oral, nasal, and fecal secretions, and can be transmitted orally and iatrogenically by contaminated allogenic stem cell products. These findings provide strong evidence that EqPV-H is a significant pathogen of horses and that management practices to prevent spread of this novel equine parvovirus should be developed.

D. Publications and Other Grant Submissions

If applicable, report publications resulting from the study, including manuscripts submitted or accepted for publication, and submissions and/or external grants resulting from the award.

We have one primary publication based on this work, which under revisions with *Emerging Microbes and Infections.* We have also leveraged some of the tools we developed for an additional farm outbreak report that is also under revisions at *Equine Veterinary Journal*. Samples collected in this study will undergo additional analysis to better understand the mechanisms of replication of EqPV-H (as proposed in a new Zweig proposal that was recently funded: "Studying the replication kinetics of equine parvovirus hepatitis (EqPV-H)"). Based on our findings here, we have recently submitted a USDA proposal for an epidemiologic investigation of EqPV-H infection in horse herds, which will be used to develop methods to control disease spread and to inform rational vaccine design. Additionally, Dr. Tomlinson has received an NIH K08 Mentored Career Development Award during these studies, which will be used to advance our understanding of the immunopathology of EqPV-H.

Principal Investigator:	Dr. Norm Ducharme
Title:	Two-day tie-back (injection laryngoplasty): proof of principle
Project Period:	1/1/17 - 12/31/19
Reporting Period:	1/1/17 - 12/31/19

A. Specific Aims of the Study and Modifications

Aim 1 (Completed): Measurement of trans-laryngeal pressures, airflow, and arytenoid angles in the 20 cadaver larynges under control, standard "suspension laryngoplasty" at 80% opening, and "injection laryngoplasty" also at 80% opening. (Phase 1)

Revised Aim 1: The start if Aim 1 was delayed many months due to the delay in obtaining import permit to receive cadaver larynges from the abattoir from Canada (Cornell vendor regulations and paperwork across countries). Ten larynges (instead of 20) were used in phase 1 for the in-vitro study. We reduced the number of larynges in Aim 1 because a) the results were so consistent in the 10 larynges that we had statistical significance proving efficacy of treatment in-vitro with 10 larynges with minimal variation, b) it became clear that we needed more cadaver larynges than planned for the multiple trials to improve the surgical approach and design of special guiding channels for injection needed of in-vivo application (Aim 3), and c) The reduced budget required some saving in some of the planned steps.

Aim 2 (Completed): Measure PMMA cure times and temperature at various dilutions used for delivering the substance to the ventral aspect of the epiglottis. (Phase 2)

Aim 2 was not modified and complete results were reported in the previous preliminary report (see under studies and results).

Aim 3 (completed): Determine efficacy by measuring tracheal pressure and arytenoid angles during exercise at 80%, 90% and 100%Hrmax prior to neurectomy (control), and every two months after "injection laryngoplasty". (Phase 3)

Revised Aim 3: Reduced budget lead to reduce the post-operative monitoring in the enrolled horses from 6 months to 3 months, and the exercise tests are scheduled at monthly interval.

Aim 4 (deleted): Assess laryngeal competency by performing tracheal wash exam prior to any treatment, after creating RLN, and one week and at month 2, 4 and 6 after treatment. (Phase 3)

Revised Aim 4: the tracheal wash exams and evaluation could not be included in the pilot study because of the reduce budget approved by the Zweig fund.

Aim 5 (completed): Recording morbidity (swelling, coughing, extrusion, etc.) of the procedures by daily physical examination, monthly endoscopic exam over a six-month period followed by gross and histopathological evaluation for assessments of local morbidity. (Phase 3)

Revised Aim 5: Because of as for Aim 3, the post-operative period was reduced from 6 to 3 months. The horses that already had surgery, were or are being currently clinically monitored. However, we have added endoscopic and ultrasonographic assessment of the surgical area performed on a weekly basis.

Aim 6 (deleted): Assess the structural integrity of the repair using robotic CT assessments. (Phase 3)

Revised Aim 6: Robotic CT evaluation could not be included in the pilot study due to reduced budget and transient unavailability of the robotic CT equipment at the Cornell Ruffian facility.

B. Summary of Scientific Findings

B1. Measure PMMA cure times and temperature at various dilutions (Aim 2):

Polymethyl methacrylate (PMMA) Bone Cement Properties Using Varying Concentrations of Powder

Introduction

Polymethyl methacrylate (PMMA) bone cement has been used successfully in human orthopedic surgery since the 1960's namely in hip replacements as a means for fastening implants to bone[1]. We routinely use PMMA in equine larynges for vocal cord bulking to reduce or eliminate tracheal aspiration in horses with dysphagia due to glottic incontinence following laryngoplasty or arytenoidectomies. Vocal fold bulking has been performed in human medicine; its first application was used by Brunings in 1911 for treatment of vocal fold paralysis[2]. In the 1960's, Teflon was originally introduced as a bulking agent, which remained the standard for 30 years[3–5]. However long-term studies revealed granulomatous reactions in some patients and therefore multiple products are been evaluated in humans[2,6–9]. One of the problems encountered with the materials used in humans is that they are designed to last around 6 months to 2 years, after which time laryngeal reinnervation has occurred. The need in horses is permanent, henceforth clinically diluted PMMA has been utilized[10]. PMMA has been used by the author as a bulking agent in horses that aspirate post-operatively from laryngoplasty or arytenoidectomy. In these horses, tracheal soiling occurs as a result of the inability to fully protect their trachea during swallowing; also known as glottic incontinence. Due to tracheal soiling, these horses often present with coughing during eating/drinking and also during exercise. Furthermore, they can result in dorsal displacement of the soft palate due to tracheal descent – caudal retraction of the larynx – as a sensory reflex mechanism to protect the airway. Injection of PMMA in the vocal fold can help offer a barrier thus preventing food contamination into the trachea. Approximately 30 horses have undergone the procedure, which has been well tolerated and successful in the standing horse.

In the current study, we aim to utilize PPMA injection as alternative to traditional laryngoplasty, by creating a supportive column that would keep the arytenoid in permanent abduction. PMMA was selected as a material because it is a solid, durable, inert substance and rarely results in inflammatory tissue reaction, and in human research survival probabilities of implanted cement have been upwards of 50 years[11]. However, the chemical reaction occurring from mixing the polymethyl methacrylate polymer with the liquid monomer is quite exothermic, yielding temperatures reportedly greater than 124 degrees Celsius[11,12]. Furthermore, bone cement was initially formulated for use in hip replacements to fasten the implant to the bone[1]. For this purpose, it is used while it is in a dough-like state. Its use in the larynx requires a viscous state to facilitate injection through an 18-gauge 3.5-inch spinal needle; furthermore, it needs to form a physical barrier (i.e., bulking) and not diffuse as a pure liquid would. It was postulated using a lower concentration of powder with the same volume of liquid would yield a substrate that could be injected and offer a longer working period before fully setting. The specific aim of this study was to determine the setting time (t_{set}), working time (t_{inject}), and peak temperature (T_{peak}) of varying concentrations of powder.

Materials and Methods

Stryker Surgical Simplex® P polymethyl methacrylate[a] radiopaque bone cement was used for this study. It is packaged into two components: a 20 ml liquid ampule (97.4% v/v methyl methacrylate monomer, 2.6% v/v N. N-dimethyl-p-toluidine, and 75+/- 15 ppm Hydroquinone) and a 40-gram packet of fine white powder (15% w/w Polymethyl methacrylate, 75% w/w Methyl methacrylate – styrene – copolymer, and 10% w/w Barium Sulfate U.P.S.). Four different concentrations of powder were investigated: 5, 6.66, 8.32,

and 10 grams of the powder polymer which corresponds to a volume of 15, 20, 25, and 30 milliliters respectively. All concentrations were mixed with a volume of 10ml liquid. For reference, the full-strength dosing is 40g packet of powder with a 20ml ampule of the liquid henceforth two experiments were run from each package.

Temperature data was collected using a 4-channel Omega data logger thermometer with 20-gauge TT insulated type K-wire thermocouples. The designated volume of bone cement was weighed and hand mixed using a bowl exposed to ambient atmosphere. Once the powder was fully dissolved, the formulation resulted in a total volume of 12-14 ml which was transferred to three 6 ml syringes with a total volume of 4 ml in each syringe. The K-wire thermocouples were inserted into the injection port of the syringes for temperature recording from the center of the volume of cement. Data acquisition was started at 2 minutes after initial combination to allow for complete mixing and transferring of bone cement. Temperature readings were recorded at 5 second intervals for approximately 30 minutes, or until the bone cement equilibrated back to room temperature after reaching peaking. The fourth probe was used to measure ambient temperature. Peak temperature and set time were determined in accordance with the specifications detailed in ISO 5833. The data was transferred to an excel document for statistical analysis.

The force data was collected using the same mixing methods. Once the bone cement was fully mixed, it was transferred to two 6 ml syringes attached to 18-gauge 3.5-inch spinal needles. A Torbal FB200 force meter was used to measure the force required to push 0.2 ml through the spinal needle. Force measurements were collected in Newtons at 1-minute intervals until the bone cement could no longer be expressed through the needle. Each experiment was run concurrently with a temperature data collection experiment providing the ambient temperature for both data sets. Each experiment was run twice. The data was consolidated to an excel document for statistical analysis.

Statistics

One-way ANOVA Kruskal-Wallis test for non-parametric data with post hoc Tukey application was used to compare differences between the selected data points ($P <= 0.05$). A linear regression analysis applied to the force data controlling for time was created with a 95% confidence interval. Data points used for statistical analysis included peak temperature, setting time (measured by the time at which the temperature is midway between ambient and peak temperature), injecting time (time able to push 0.2 ml through the syringe) and force measurements.

Results

In accordance with ISO 5833, each experiment was run at 23° C +/- 1; between two experiments a peak temperature within a 10°C range and a setting time within 1 minute is required to accept the experiment. If there is a discrepancy, the experiment must be run in duplicate. Henceforth, each concentration was run 4 times apart from 8.32g of powder, which only needed to be run twice. The average peak temperatures (Table 2) did not increase on a linear scale and were 57.4 ±2.5, 83.5 ±4, 99.2±5.1 and 98.5±4.3°C respectively for 5, 6.66, 8.32, and 10 grams of powder. There was significant difference between all concentrations except for 8.32g of powder and 10g of powder ($P \leq 0.05$). The average setting times (Table 2) were 26.1 ±3.6, 20.4±3.3, 17.1±0.9, and 14.0±1.9 minutes for the same progression of concentrations. Based on the graphical information, the setting times decreased as the concentration increased. The setting time for 5g of powder was not significantly different from 6.66g. There was no significant difference between 6.66 and 8.32g of powder and no significant difference between 8.32 and 10g of powder, however there was significant difference between 6.66g and 10g of powder. The average injecting times (Table 2) decreased as concentrations increased and were as follows 16.5±0.7, 10.5±3.5, 6.5±0.7, and 4±1.4 for 5, 6.66, 8.32, and 10 grams of powder. There was no significant difference between 5g and 6g of powder. No significant difference between 6.66, 8.32, or 10g of powder. See Table 1 for complete results. A linear regression analysis yielded a significant difference between the intercepts calculated from the force data

acquired at 1-minute intervals indicating a significant difference in the force required to inject as it changes over time.

Table 1 concentration of powder used and resulting values of the properties of the cement

Concentration of powder (g)	T_{peak} (C°)	t_{set} (min)	t_{inject} (min)
5	60.4[a]	30.6[a]	17[a]
5	57.2[a]	27.4[a]	16[a]
5	54.5[a]	22.8[a]	
5	57.6[a]	23.6[a]	
6.66	77.7[b,c]	16.1[a,b]	8[a,b]
6.66	84.3[b,c]	23.8[a,b]	13[a,b]
6.66	86.6[b,c]	20[a,b]	
6.66	85.5[b,c]	21.8[a,b]	
8.32	102.8[b,c,d]	17.7[b,c]	7[b,c]
8.32	95.6[b,c,d]	16.4[b,c]	6[b,c]
10	92.6[c,d]	11.7[c]	3[b,c]
10	98.1[c,d]	14.1[c]	5[b,c]
10	102.6[c,d]	13.6[c]	
10	100.7[c,d]	16.4[c]	

[a,b,c] same superscript are not significantly different from each other

Table 2 PMMA property averages

Concentration of Powder (g)	Peak temperature (C°)	Setting Time (min)	Injection Time (min)
5	57.4 ±2.5	26.1 ±3.6	16.5±0.7
6.66	83.5 ±4	20.4±3.3	10.5±3.5
8.32	99.2±5.1	17.1±0.9	6.5±0.7
10	98.5±4.3	14.0±1.9	4±1.4

Discussion

The International Organization for Standardization for acrylic resin cements used for implant surgery defines the peak temperature as the maximum temperature reached during an experiment, and the setting time as the mid-way point between ambient temperature and peak temperature[13]. Injection time has not been defined as bone cement is traditionally used in a dough state, and as such the doughing-time is defined as the time it takes for bone cement to no longer stick to a gloved finger[13]. For this study, the injection time was defined as the time in which 0.2 ml could be injected from a 6 ml luer-lock syringe connected to an 18-gauge, 3.5-inch spinal needle. This syringe, needle, and volume combination was selected based on the author's experience and use in a clinical setting for PMMA injection into the equine larynx, with 0.2 ml being a standard, repeatable volume to inject in *in vitro* testing.

In human medicine, the approach for vocal fold bulking is either through oral approach or the cricothyroid membrane; the latter approach can also be carried out in the equine species using an 18-gauge 3.5-inch spinal needle[2]. In horses, the procedure can be performed under standing sedation in combination with the use of local anesthetic. Because of this, a concentration of bone cement that minimizes peak temperature, minimizes the time to set, and maximizes the injection time is desirable, as the sedated horse can be unpredictable compared to a patient under general anesthesia.

Based on the results, with clinical interpretation, the concentration 8.32 grams (25ml) powder in 10ml solvent appears to be the best suited concentration for its application in the equine larynx. There are no statistically significant differences between peak temperatures, setting time, or injection time for 8.32 and 10 grams of powder; nevertheless, in a clinical setting, the difference between 4 minutes and 6.5 minutes to inject can be significant. Henceforth, using 10 grams of powder has an increased risk of setting before the

desired volume of cement had been injected. When comparing 6.66 and 8.32 grams of powder, there are also no statistically significant differences between peak temperature, setting time, or injection time. The average time to set is approximately 20 minutes and 17 minutes for 6.66 and 8 grams of powder respectively. Again, in a clinical setting, the minimum amount of time for the PMMA to set may result in a more desirable outcome due to the unpredictable nature of a sedated horse. Henceforth, it can be concluded 8.32 grams of powder maximizes the amount of time to inject and minimizes the time to set; peak temperatures being equal. Additionally, the injection requires the bone cement to be in a viscous state. Though PMMA rheological properties were not specifically measured in this experiment, it is the authors' experience 8.32g of powder has sufficient augmenting or bulking effect when injected submucosally, as compared to more dilute concentrations which tend to diffuse under the submucosa.

Peak temperatures in *in vitro* testing have been reported in the literature as upwards of 124°C. It is unclear why thermal necrosis and sloughing of the mucosa does not occur. However, in one study investigating thermal necrosis with PMMA in femoral head implants, *in vivo* intra-operative temperatures reached 54.12°C at the cement/bone interface, but 110.2°C in the mixing bowl; in which the bone interface temperature is not likely to result in thermal necrosis[14]. It is speculated this is a reflection of the body's ability to conduct heat, likely through perfusion[14].

Ambient temperature has been proven to alter the handling, thermal, and physical properties of PMMA[15]. As ambient temperature rises, the exothermic reaction is accelerated resulting in more rapid setting times, in turn decreasing working times; interestingly peak temperatures remain unchanged[15]. Considering this factor also aided in the selection of 8.32 grams of powder, as an increased concentration may set more rapidly precluding complete injection.

In conclusion, it is our opinion a dilution of 8.32 grams (25ml) of powder to 10 milliliters of solvent is the most favorable concentration for injection into the equine larynx.

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B2. Measurement of trans-laryngeal pressures, airflow, and arytenoid angles in the 20 cadaver larynges under control, standard "suspension laryngoplasty" at 80% opening, and "injection laryngoplasty" also at 80% opening. (Aim 1)

Injection (i.e. Bone Cement) Laryngoplasty – Cadaver Phase

Materials and methods

A total of 10 larynges were utilized for the in-vitro experiments. Each larynx was dissected to remove the cricopharyngeus muscle and esophagus and the trachea was sectioned at the 3rd or 4th tracheal ring. The experiments were conducted in vitro utilizing a flow chamber as previously described (Cheetham et al, 2008). The chamber is constituted by a closed wooden box (30x 15x 15cm) with a Plexiglas lid. The box has two opening (cranial and caudal) made by PVC pipe (50mm internal diameter). The caudal opening is connected externally to a vacuum system (two 16-gallon vacuum cleaners) attached in parallel with a rheostat used to generate negative pressure and so produce an inspiratory flow that could be varied. The PVC pipe contains a cycling valve (PVC disk, 1mm radial clearance) with contacting valve seals at the fully closed position to occlude airflow. The valve cycled at 2 Hz producing intermittent airflow, to simulate the breathing frequency reached by horses during intense exercise. The vacuum system was connected in line to a rotameter (KDG Flowmeters, West Sussex UK) to measure the liter/min flow obtained by the vacuum system. Within the box, the PCV pipe is connected to the caudal aspect of the tracheal portion of the cadaver larynx by a clamp. The tip of the epiglottis is secured to a wooden board with a stainless-steel pin to prevent retroversion. Two Teflon catheters (1.3mm ID, 1.9mm OD, Cole-Palmer International, Chicago, IL) were placed through the cranial opening so that one catheter lay cranial to the larynx (simulating pharyngeal area), and the second one within the trachea. The catheters were then attached to differential pressure transducers (Celesco LCVR, Celesco Products Inc., Canoga Park, CA) referenced to atmospheric pressure and calibrated from -70 to 70mmHg using a manometer. Catheters were in phase from 1 to 20Hz. An endoscope was passed through the cranial opening to lay cranial to the larynx and visualize the arytenoids and glottal opening.

Each larynx was used to create 3 distinct condition in order: 1) bilateral traditional laryngoplasty (to simulate normal laryngeal function), 2) left sided hemiplegia (by removing the left sided laryngoplasty), and 3) injection laryngoplasty.

For each condition, the larynx was placed in the box, secured with stainless-steel pins and clamps to the PCV and underlying board. The vacuum system was activated to create and maintain a flow of 30L/sec. Once the flow was steady, recordings of the endoscopic images of the larynx and the pharyngeal and tracheal pressures were collected for 1 minute. The test was initially performed on the larynx with bilateral traditional laryngoplasty, thereafter the left laryngoplasty suture was transected and removed, to simulate left sided paresis. The last condition created in each larynx was a left sided injection laryngoplasty. An incision was made in the ventral thyroid cartilage, at the level of the vocal fold, approximately 5mm lateral to the thyroid notch. A blunt periosteal elevator was used to tunnel, submucosally, a path up to the ventral aspect of the corniculate process of the left arytenoid. A plastic tube (4mm outer diameter) was tunneled to reach the arytenoid. A suture anchor was advanced into the plastic tubing and anchored into the corniculate process. Bone cement was injected into the tubing, while pushing the arytenoid in abduction, at a dilution of 25ml (8.32g) powder in 10 ml diluent. The column of bone cement was lateralized by placing a suture around the column of cement through the thyroid cartilage.

Data analysis

Descriptive data are expressed as means with standard deviation (SD). Endoscopic images and airway pressures recorded at the end of the one-minute test for each condition, ie. traditional laryngoplasty, left side paresis and injection laryngoplasty, were analyzed. Endoscopic images of the rima glottidis were captured from the recordings using editing software (Video Wizard, Womble Multimedia, CA, USA) to measure arytenoid abduction. Image frames corresponding to three inspiratory phases during flow cycling were evaluated and averaged. Breathing phases were identified using synchronized airway pressure traces overlying the video recordings. The degree of arytenoid abduction was measured drawing a line connecting the dorsal- and ventral-most points of the rima glottidis. This line is then extended dorsally for a distance of one third of the dorso-ventral height of the rima glottis. A tangential line to the arytenoid cartilages is drawn, and the angle between the dorsoventral line and the tangential line is measured. Airway pressures peaks recorded for 8 consecutive seconds at the end of the 1-minute test were averaged for each condition. The line connecting the dorsal- and ventral-most points of the rima glottidis and the medial border of the corniculate process and vocal cords were used to draw and measure the cross-sectional area of each side of the rima glottis; the ratio between the left and right portion of the glottis was calculated for each condition (XSA L/R).

A mixed-effect model (with laryngeal specimen as a random effect and experimental condition as the fixed effect) was fitted to the data for each outcome measure (Pharyngeal pressure, tracheal pressure, arytenoid angle, XSA (L/R)) in both the static and cycling testing states. The rationale for using a mixed model analysis of the data is to control for potential clustering of observations by specimen that could be due to factors that affect the response of the specimen other than experimental ones. A Tukey's post hoc test was used to make multiple comparisons between the two conditions to test whether injection or standard laryngoplasty resists the forces produced by physiological laryngeal flows and pressures. Statistical analysis was performed using JMP (SAS Institute, Cary, North Carolina, USA). Significance was set at $p<0.05$ throughout.

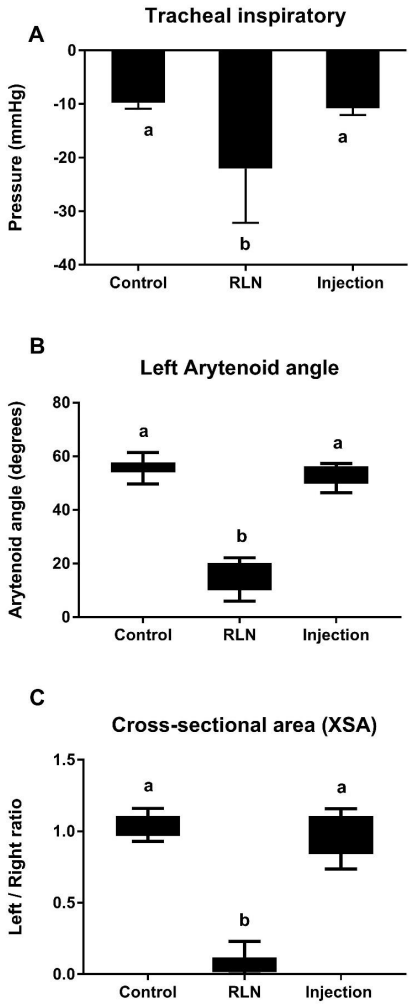
Results

The bilateral traditional laryngoplasty, simulating normal laryngeal function, was considered as our control condition. Table 1 summarizes the data recorded for each condition.

On mixed effect model, controlling for the laryngeal specimen, a significant effect of the condition was detected for all the outcome measures taken into consideration (Pharyngeal pressure, tracheal pressure, arytenoid angle, XSA (L/R)). On Tukey's post hoc test, comparisons between the 3 conditions showed a significant difference between the hemiparesis (RLN) and the two treatments (traditional laryngoplasty and injection laryngoplasty) ($p<0.001$; Fig 1). No significant difference instead was detected between the performances of the two treatments ($p>0.2$).

Table 1. In vitro performance of injection laryngoplasty against cyclic airflow, compared to traditional laryngoplasty (control) and left sided arytenoid paresis (RLN)

	Pharyngeal (I) (mmHg)	Tracheal (I) (mmHg)	XSA (L/R)	Left arytenoid angle (degree)
Control	-5.7 ± 0.77	-9.77 ± 1.09	1.04 ±0.08	55.89 ± 3.25
RLN	-3.02 ± 1.26	-22.05 ± 10.11	0.07 ± 0.07	14.41 ± 5.6
Bone Cement	-5.86 ± 0.87	-10.78 ± 1.27	0.99 ± 0.14	52.83 ± 3.64
Data are mean ± standard deviation.				



Discussion

We hypothesized that by supporting the arytenoid cartilage by strategic placement of PMMA into the ipsilateral vocal cord would allow treatment of both vocal cord and arytenoid cartilage collapse during exercise. In this phase of the study we tested the impact on airway mechanics of this procedure (injection laryngoplasty) compared to the standard procedure (traditional laryngoplasty) in cadaver larynges.

The results we obtained proved that the injection laryngoplasty can ameliorate the arytenoid abduction, glottic area and tracheal airway pressure compared to the condition of left sided paresis. Moreover, we demonstrated that in-vitro, not only the performance of the injection laryngoplasty are similar to the suspension (traditional) laryngoplasty, but also that there is no significant difference regarding the airway mechanics between the two surgical approaches.

These results, confirming our initial hypothesis, gives support to translate this in-vitro model to an in-vivo study, to evaluate morbidity, long-term stability and performance during exercise of the injection laryngoplasty.

Figure 1. Tracheal inspiratory pressure, left arytenoid angle and glottis cross sectional area of equine cadaver larynx tested into a flow chamber. In (A), simulation of left sided hemiplegia (RLN) induced a significant decrease in the tracheal pressure compared to control, while the injection laryngoplasty returned the pressure to normal values. In (B), simulation of left sided hemiplegia (RLN) induced collapse of the left arytenoid during the test. The injection laryngoplasty re-created an abduction angle not significantly different from the traditional laryngoplatsy (Control). In (C), bilateral traditional laryngoplasty (Control) maintained a symmetrically opened rima glottis, with the two halves of the rima glottis showing a left to right ratio around 1. Simulation of left sided hemiplegia (RLN) caused arytenoid and vocal cord collapse with significant reduction of the left glottic area, indicated by a left/ratio ratio approaching zero. The injection laryngoplasty improved the left glottic

area, making it similar to the right one, with a ratio not significantly different from the control condition. Data are mean ± SD. Different letters indicate statistically significant differences between conditions.

B.3 Recording morbidity (swelling, coughing, extrusion, etc.) of the procedures by daily physical examination, monthly endoscopic exam over a six-month period followed by gross and histopathological evaluation for assessments of local morbidity. Determine efficacy by measuring tracheal pressure and arytenoid angles during exercise at 80%, 90% and 100%Hrmax prior to neurectomy (control), and every two months after "injection laryngoplasty". (Aim 3 and 5).

Injection (i.e. Bone Cement) Laryngoplasty – *In vivo* study (Phase 3)
***Study design*.** The goals of Phase 3 were to assess the feasibility, effectiveness, stability, and morbidity of injection laryngoplasty with experimentally created laryngeal hemiplegia. Horses with normal laryngeal structure and function were used in the study. The horse laryngeal function was evaluated through endoscopy before surgery, to establish their native function, after inducing left laryngeal hemiparesis, and following injection laryngoplasty. After injection laryngoplasty, the horses were examined daily, to monitor for healing progression and possible development of complications. Endoscopy and laryngeal ultrasound were performed on a weekly basis to verify laryngeal structures and perilaryngeal tissue response to the bone cement injection. Based on the short-term surgical outcome, the horses were euthanized to perform a laryngeal dissection to determine local reaction to the material on the larynx and surrounding structures.

Materials and Methods
Animals. Horses donated to Cornell University were enrolled in the study if in good health condition, with normal laryngeal function assessed through upper airway endoscopy at rest and during exercise (Havemeyer scale grade I-II/IV at rest, grade A during exercise) (Ducharme, 2003).

Dynamic testing. An incremental standardized exercise test was performed to evaluate arytenoid function under increasing inspiratory negative pressure loads. Horses underwent a daily training on high-speed treadmill, and when adequately fit, they underwent a standardized strenuous incremental exercise. Because of the numerous delays in the study progression, it was decided to modify the incremental exercise so that each horse would have to perform a single test for each time point (prior to laryngeal hemiparesis, with laryngeal hemiparesis and after injection laryngoplasty). Instead of performing an incremental strenuous exercise to determine the maximal heart rate and calculate the speed inducing 50, 80, 90 and 100%hrm, each horse was subjected only to the strenuous exercise while recording videoendoscopy and airway pressures. During the trial the treadmill was started at time 0, accelerated to 4 m/sec; the treadmill was then inclined to a 10% slope, accelerated to 6 m/sec and kept at that speed for 1 min. Each subsequent minute, the treadmill was accelerated by 1 m/sec until the horse was no longer capable of maintaining position near the front of the treadmill. During the trial, we recorded simultaneously heart rate, upper airway videoendoscopy, pharyngeal and tracheal airway pressures, and accelerometer measurements. Laryngeal function was recorded using a wire-less videoendoscope (Optomed) placed into the nasopharynx via the right ventral nasal meatus. Two Teflon® catheters (1.3 mm I.D., Neoflon, Cole-Parmer, Chicago, IL 60061) were placed under endoscopic guide through the left ventral nasal meatus so the ports would lie respectively in the pharynx and in the trachea. The catheters were attached to differential pressure transducers (Celesco LCVR, Celesco Transducers Products Inc, Canoga Park, CA) referenced to atmospheric pressure and calibrated from -70 to 70 mmHg (Nielan, 1992). Peak inspiratory and expiratory pressure were measured in the last 20 seconds of each speed interval. Endoscopic images of the rima glottis were captured from the recordings at the end of each interval using editing software (Video Wizard, Womble Multimedia, CA, USA) and the degree of arytenoid abduction and glottic area were measured (Herholz, 1997; Cheetham, 2011).

Induction of laryngeal hemiplegia

- *Recurrent laryngeal nerve (RLn) transection.* Animals were restrained in a stock and sedated with detomidine in intravenous constant rate infusion. The left mid-cervical area was clipped and prepared for sterile surgery; thereafter a local anesthesia of the skin was performed injecting lidocaine 2% (10ml) along the jugular groove. A 5cm incision was made, and the left RLn was exposed through blunt dissection of the subcutaneous tissues. The recurrent laryngeal nerve was isolated from the contiguous carotid sheath, and its identity was confirmed using a blunt bipolar stimulating tool (Bipolar probe, Neurosign, UK) connected to a portable stimulation device using 1–5 mA, a frequency of 1–3 Hz and pulse duration of 1 ms (CLEO Nerve monitoring, Inomed GmbH, Germany). RLn stimulation was confirmed through the consequent left arytenoid abduction using an endoscope passed through the right ventral nasal meatus to visualize the larynx. The RLn was then transected and ligated, following resection of a 2cm section. The subcutaneous tissues of the surgical incision were re-apposed using 2/0 monocryl in a simple continuous pattern and skin closed with staples.

- *Recurrent laryngeal nerve (RLn) block.* After the first two horses, the enrollment of the other horses happened with delay, and because of time restrain in conducting the study, it was decided to simulate the RLN condition when needed, by inducing left sided hemiparesis performing RLn anesthetic block. This approach allowed to remove from the horses timeline the post-operative resting time necessary after the RLn transection. For the RLn block, the horses were restrained in a stock, and the left mid-cervical area was clipped and prepared for sterile procedure; thereafter a local anesthesia of the skin was performed injecting lidocaine 2% (2ml). A stimulating injectable 22G needle (UniPlex Nanoline cannula, PAJUNK® GmbH, Germany), was inserted dorsal to the jugular vein and perpendicular to the skin. Eletrical stimulation at 1mA and 100 µsec (Innervator 232; Fisher & Paykel Healthcare) was then applied to produce stimulation of the RLn while the arytenoid response was monitored through an endoscope passed via the right ventral nasal meatus. After confirmation of left arytenoid response to the RLn stimulation, 5ml lidocaine 2% was injected through the needle to anesthetize the RLn. The RLn block was performed before the incremental exercise test on high-speed treadmill when gathering data regarding the left hemiparesis condition.

Surgical procedure (injection laryngoplasty)

Horses were restrained in a stock and sedated with detomidine in intravenous constant rate infusion. The laryngeal area was clipped, aseptically prepared, and draped in sterile fashion to isolate the ventral and left larynx. An 8 cm incision was made ventral to the linguofacial vein and the larynx dissected out. A 1cm incision was made in the thyroid cartilage, left of the thyroid notch. A blunt periosteal elevator was used to create a path to the vocal process of the arytenoid cartilage. Silicon-coated plastic tubing was passed into the tunnel to the vocal process and a 3.5 suture anchor was inserted into the tubing. Attempts were made to pass the suture anchor into the corniculate, however the anchor broke through the mucosa and the anchor was abandoned. A 3.5 mm suture anchor was passed through the muscular process in a caudoventral to rostrodorsal fashion. The suture was anchored to the thyroid cartilage laterally Stryker bone cement was injected into the tubing placed to the vocal process and the arytenoid held into abduction with a tenaculum until it hardened.

The incision was closed- 1 monocryl was used to tack the ventral larynx to the subcutaneous tissues, minimizing deadspace. The fascia of the linuofacial vein was apposed to the omohyoidius with 0 monocyrl in a simple continuous pattern. Subcutaneous tissues were closed with 2-0 monocryl in a simple continuous followed by staples in the skin.

Results

Horses. Four adult horses of different breed (2 Thoroughbred, 1 Standardbred and one Percheron cross) were included in this study; one castrated male and three females, median age 12 years (range 8-18), median weight 534 kg (range 450-673 kg) with normal laryngeal function (Havemayer grade I or II at rest, grade A at exercise).

Surgical procedure. Performing the surgery on the first three horses helped identifying several issues that did not manifest during the in-vitro phase.

- Advancement of a suture anchor in the corniculate process of a standing, moving horse without mucosal penetration proved to be much more difficult than originally anticipated. This approach was abandoned. Through further in-vitro tests, it was determined adequate abduction could be achieved with suture anchor placement into the muscular process alone with the column of bone cement supporting the arytenoid from the muscular process with a single anchor. An 8G Jamshidi needle was used to facilitate suture anchor placement into the muscular process. Following this discovery, it was determined the lateralization of the column of bone cement was unnecessary.
- Abduction of the arytenoid while the bone cement hardened could be achieved with an endotracheal tube passed through the ventral meatus to the level of the larynx with inflation of the endotracheal cuff to abduct the larynx. This abducted the arytenoid in a controlled fashion while allowing the horse to be able to breath. This was first tested in-vitro prior to study horses. In-vivo, a sample of bone cement was kept over the surgery table to monitor for the setting time, and once the cement was deemed adequately solidified, the endotracheal tube cuff was deflated and the tube removed. The suture connected with the suture anchor was passed twice around the ventral border of the thyroid cartilage and knotted upon solidification of the bone cement.
- A ventral approach through a laryngotomy was sufficient for visualization and required much less dissection than the lateral approach. The laryngotomy incision was closed- 1 monocryl was used to tack the ventral larynx to the subcutaneous tissues, minimizing deadspace. Subcutaneous tissues were closed with 2-0 monocryl in a simple continuous suture followed by staples on the skin.
- Several of the more minor steps were deemed to be redundant: 1) incising into the thyroid cartilage was replaced with direct penetration with the jamshidi needle; 2) tunneling with the blunt periosteal elevator was unnecessary; the jamshidi was passed through the thyroid cartilage and advanced to the muscular process; 3) bone cement could be injected directly into the jamshidi needle, eliminating the use of the plastic tubing making the procedure more streamlined.
- The endoscopic visualization of the larynx, would allow for monitoring the insertion of the needle along the vocal cord but would not allow to establish the exact location of the needle tip in respect to the arytenoid body and muscular process and so the location of the most proximal end of the cement column after injection. For this reason, the following surgeries were conducted with ultrasonographic support, to ensure the needle tip was located toward the muscular process, and not pointing too caudal toward the cricoid cartilage. The insertion of the Jamshidi needle along the vocal cord, because of its size and the proximity and irregular shape of the ventricle, caused the laceration of the ventricle in its most proximal portion. This complication did not manifest during surgery, possibly due to the location, but several days after, when the mucosa laceration enlarged and exposed the underlying bone cement. To lower the risk of ventricle damage, which could lead with time to secondary infection, the surgical approach was optimized again through in-vitro trials, during which it was investigated which approach would allow the same degree of abduction avoiding the area nearby the ventricle. Based on these in vitro trials, during the last surgery performed, the needle was inserted in a more caudal position along the ventral edge of the thyroid cartilage, and then advanced along its medial aspect.
- Several factors affected the continuity of the bone cement injection during the first few surgeries, like the presence of the suture in the jamshidi needle and filling the endotracheal tube to create abduction after starting to inject the bone cement. The discontinuity caused irregular width of the bone cement column that after few days broke in the thinnest/weakest spots.
- The bone cement column in the first two horses did not resulted in an optimal position that could be maintained after the end of surgery. The first horse developed moderate swelling over the bone cement column, partially obstructing the left hemilarynx, and due to perforation of the laryngeal mucosa while manipulating the screw anchor, a secondary infection of the surgical site developed after few days, attributed to the pharyngeal microflora penetrating the mucosal defect. Changes in the technique have resolved this complication in the other horses treated. The next 3 horses obtained the desired grade 2

abduction (circa 50-80º to sagittal plane) (Dixon et al, 2003) after the injection laryngoplasty, but lost the abduction 7-10 day after surgery. On post-mortem dissection the cement column, despite good location, resulted to have broken portion of the PMMA.
- The final horse used for the *in-vivo* study showed the injection laryngoplasty failed due to cement fracture in multiple locations upon dissection of the larynx. The fractured pieces of PMMA were infiltrated with fibrous tissue suggesting early failure of the column of cement, which correlates with endoscopic findings. The anchor was placed in a suitable location, suggesting the approach for injection laryngoplasty is good and repeatably achievable *in-vivo*. However, PMMA revealed to be the wrong product; PMMA is very strong in compressive forces but weak in shear. The cyclical loading from repetitive adduction encountered with swallowing, and respiration during exercise loaded the cement in shear resulting in failure at multiple locations.

Morbidity. The bone cement column in the first two horses resulted in a suboptimal position that did not allow maintaining abduction already at the end of surgery. The first horse developed moderate swelling over the bone cement column, partially obstructing the left hemilarynx, and had a secondary infection of the surgical site developed after few days, attributed to the pharyngeal microflora penetrating the mucosal defect. The second two horses obtained at the end of the surgery, grade 2 abduction (circa 50-80º to sagittal plane) (Dixon et al, 2003) after the injection laryngoplasty, but the days after surgery the abduction dropped to 45º. On post-mortem dissection the cement columns, despite a good location, resulted to have broken in one of the thin portions of cement possibly created by the non-continuous injection of the cement in one horse, and at multiple locations in the second horse.

Performance during exercise. The first horse was not tested during exercise after the injection laryngoplasty due to the swelling and infection in the laryngeal area. The second horse, despite a low surgical laryngeal abduction, showed a steady vocal cord that did not collapse during exercise, thanks to the bone cement keeping the vocal cord and ventricle in a lateral and fixed position. The third horse showed collapse of the corniculate process and vocal cord, because the bone cement column was broken in multiple spots along its course, so becoming unsteady even to support the vocal cord. The fourth horse had partial collapse of the arytenoid during exercise, though the vocal cord was stable and stayed lateralized.

C. Significance

The results from the in vitro phases that we completed, indicate that from a mechanical point of view the injection laryngoplasty could represent a valid alternative to the traditional laryngoplasty.

The in-vivo phase allowed identifying and troubleshooting several issues, most significantly, the material used for injection being too weak in shear forces (i.e cycling during swallowing) resulting in failure of the cement column; we are returning to in-vitro testing with different materials to optimize the in-vivo surgical approach.

D. Publications and Other Grant Submissions

1. Management of post-operative dysphagia following prosthetic laryngoplasty or arytenoidectomy Lauren Luedke, DVM, Hussni Mohammed, PhD, Norm Ducharme, DVM, MSc, DACVS
   Vet Surg. 2020 Apr;49(3):529-539.
2. Polymethyl methacrylate (PMMA) Bone Cement Properties Using Varying Concentrations of Powder Lauren Luedke, DVM, Norm Ducharme, DVM, MSc, DACVS, Hussni Mohammed, PhD
   Manuscript being submitted for publication: Frontiers in Veterinary Medicine

Principal Investigator:	Dr. Susan Fubini
Title:	The Relationship between Obesity and Post-Operative Incisional Infections Following Abdominal Surgery in the Horse
Project Period:	1/1/17 - 6/30/20
Reporting Period:	1/1/19 – 12/31/19

The first is a retrospective study of abdominal surgery cases from Cornell University and The University of Georgia looking at the incidence of incisional infection in horses and if obesity is a risk factor. Data analysis on 242 cases showed there was a trend towards an association between incisional infection and obesity. In order to strengthen our data, and in consultation with Dr. Hayes, we added the last year of cases (until August 2018) at both Universities. Data collection is complete, analysis done, and manuscript written. The paper has been reviewed for the Equine Veterinary Journal, changes made, and re-submitted. Dr. Hill, now in specialty practice in Littleton Colorado, will present the results in December at the 2019 American Association of Equine Practitioners meeting.

The second project was planned to be a prospective study on the incidence of incisional infection following abdominal (colic) surgery. We were planning to measure parameters that are associated with body mass such as adipokines in an attempt to provide more objective data. Like many schools and referral practices our surgical colic caseload has dropped dramatically. As of August 1, 2018 we only had 20 horses enrolled in the study and we were aiming for 100. For this reason, as mentioned, we added those cases to the retrospective study, along with cases during the same time frame from the University of Georgia. We have found it to be nearly impossible to involve other referral centers or academic institutions when measurements are needed and blood samples taken and transported. For these reasons we are terminating this work and returning funds to the Zweig Committee.

Principal Investigator:	Dr. Philippa Johnson
Title:	Equine brain white matter: A comparative tractography and gross dissection study
Project Period:	12/1/16 - 11/30/19
Reporting Period:	12/1/16 - 11/30/19

A. Specific Aims of the Study and Modifications

Specific Aims: The specific aims were altered

Aim 1. Create and make freely available a high-resolution stereotaxic population average brain atlas for the equine including T1-weighted brain template, tissue segmentation maps (TSPs) for white matter, grey matter and cerebrospinal fluid, and segmented priors of the subcortical brain structures.

Aim 2. Document mean volume metrics for the whole brain, grey matter and white matter and assess the effect of age and laterality on tissue volumes

B. Summary of Scientific Findings

Purpose. There is growing interest in the horse for behavioral, neuroanatomic and neuroscientific research due to its large and complex brain, cognitive abilities and long lifespan making it neurologically interesting and a potential large animal model for several neuropsychological diseases. Magnetic resonance imaging is a powerful neuroscientific research tool that can be performed in-vivo, with adapted equine facilities, or ex-vivo in the research setting. The brain atlas is a fundamental resource for neuroimaging research, and have been created for a multitude animal models, however none currently exist for the equine brain. In this study we document the creation of a high-resolution stereotaxic population average brain atlas of the equine.

Methods. The atlas was generated from nine unfixed equine cadaver brains imaged within 4 hours of euthanasia in a 3-tesla MRI. The atlas was generated using linear and non-linear registration methods and quality assessed using signal and contrast to noise calculations. Tissue segmentation maps (TSMs) for white matter, grey matter and cerebrospinal fluid, were generated and manually segmented anatomic priors created for multiple subcortical brain structures.

Results. The resulting atlas was validated and correlated to gross anatomical specimens and is made freely available at as an online resource for researchers (https://doi.org/10.7298/cyrs-7b51.2). The mean volume metrics for the whole brain, grey matter and white matter for the included subjects were documented and the effect of age and laterality assessed. Alterations in brain volume in relation to age were identified, though these variables were not found to be significantly correlated. All subjects had higher whole brain, grey matter and white matter volumes on the right side, consistent with the well documented right forebrain dominance of horses.

Conclusions. This atlas provides an important tool for automated processing in equine and translational neuroimaging research.

C. Significance

Emphasize the significance of the findings and their potential impact.

This is the first and only stereotactic brain atlas available for the horse and provides a valuable resource for future advanced neuroimaging in the equine. The atlas has applications for anatomic localization in the equine brain as well as MRI data registration and segmentation. This is also the first MRI evaluation of the equine

brain that has looked at laterality within the brain and our findings support that described behaviorally in the horse.

D. Publications and other grant submissions

Report publications resulting from the study, including manuscripts submitted or accepted for publication, and submissions and/or external grants resulting from the award.

(Johnson et al., 2019) Johnson, P. J., Janvier, V., Luh, W. M., FitzMaurice, M., Southard, T., and Barry, E. F. (2019). Equine Stereotaxtic Population Average Brain Atlas With Neuroanatomic Correlation. *Front. Neuroanat.* doi:10.3389/fnana.2019.00089.

E. Resident's Assessment

Research Development: Briefly describe your involvement in activities throughout the project to increase your research skills. Include, for example, formal course work, informal instruction in specific research skills, scientific seminars and meetings, training in the responsible conduct of research, or visits to other laboratories.

Other Activities: Briefly describe your involvement in activities other than research and research training during the project. Describe activities such as teaching, clinical care, service on advisory groups or committees, and administrative activities.

F. Mentor's Report

Provide a mentor's statement assessing the Resident's progress and performance throughout the project, both in research productivity and in terms of research understanding.

Dr. Janvier has worked diligently on this project. We had to change the focus of the project part way through due to data processing pipeline issues. The final project used structural data collected at the time of diffusion tensor imaging and allowed us to create the described atlas. This layered on Dr. Janvier's imaging knowledge providing him with advanced MRI knowledge. Dr. Janvier presented a poster at the ACVIM forum in Phoenix Arizona in June 2019.

Principal Investigator:	Dr. Hussni O. Mohammed
Title:	Factors predispose to musculoskeletal injuries and catastrophic events in racing horses
Project Period:	1/1/16 - 12/31/19
Reporting Period:	1/1/16 - 12/31/19

A. Specific Aims of the Study and Modifications

Specific Aims: If the aims have not been modified, state so. If they have been modified, provide the revised aims and the reason for the modification.

1. First, determine the incidence and nature of MIs and CEs among horses racing at New York Thoroughbred Racing Tracks in collaboration with the veterinary staff at NYRA and Finger Lakes Thoroughbred tracks.
2. Second, identify modifiable factors that predispose horses to the risk of particular MIs or CEs including intrinsic and environmental factors.
3. Third, investigate the the potential use of pNF-H as a biological marker and predictor of the risk of MIs and CEs for the purpose of making management recommendations to mitigate their adverse consequences while accounting for the potential role of oxidative stress.

B. Summary of Scientific Findings

Describe the studies directed toward the specific aims and the positive and negative results obtained. If applicable, address any changes to the innovative potential of the project. If technical problems were encountered in carrying out this project, describe how your approach was modified.

We have carried out retrospective and prospective studies to determine the incidence of musculoskeletal injuries (MIs) and catastrophic events (CEs) among Thoroughbred (TB) racehorses. Our initial focus was on the bilateral proximal sesamoid bone (BPSB) fracture, (Palmer et al., 2017). Musculoskeletal fractures comprised 79% of the fatalities; cardiopulmonary conditions accounted for 12% of the fatalities. Other causes of death included gastrointestinal (3%), respiratory (5%), and central nervous system (2%) conditions. Regarding the MIs and CEs, fetlock failure represented 50% of the musculoskeletal fatalities. These findings, used in conjunction with a comprehensive mortality review process and regulatory reform, have contributed to a significant reduction of the incidence of TB racing fatalities at New York Racing Association (NYRA) racetracks during the period of this review.

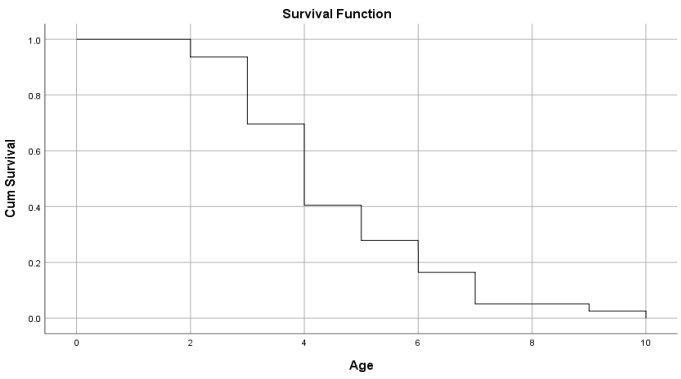


Figure 1. The incidence/probability of BPSB past certain age of the horse

In the retrospective component of the study we investigated the incidence of BPSB among horses raced during the seasons of 2010 to 2016 (Palmer et al., 2020). Figure 1 shows the survival curve for the incidence of PBSP past certain age of the horse. The median age to experience CE incidence of BPSP was 4 years. On the other hand, Figure 2 shows the survival curve for the incidence of PBSP past certain number of life starts. The median number of lifetime starts to be experiencing CEs of BPSB fracture was 13 number of starts.

Furthermore, we identified 63 additional putative risk factors and used a systematic data analysis approach to evaluate the association between these factors and the risk of (BPSB) fracture (Palmer et al., 2020). Identified indigenous and management factors associated with increased risk included: intact male, age at race, average starts per year, career training breaks, and the number of race in the past 6 years. Whereas, the number of weeks in training was the only factor that demonstrated to be associated with decreased risk of MIs and CEs incidences. Additionally, we assessed the impact of the pre-race veterinary exam on the risk of BPSB. Using a similar systematic data analysis approach, we found that the risk of BPSB fracture increased with the number of positive Pre-race exam findings (Palmer et al., 2019).

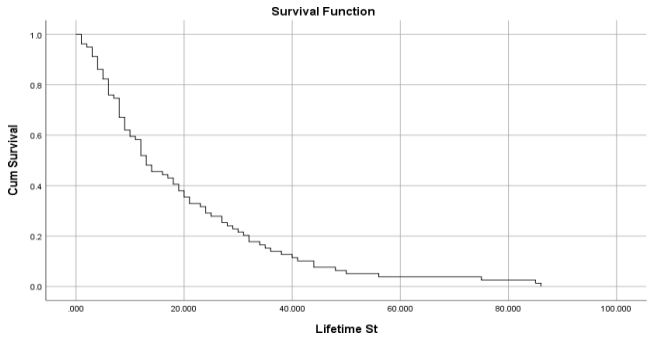


Figure 2. The incidence/probability of BPSB past certain number of life time starts

We also continue to conduct studies of the incidence of both MIs and CEs among TB horses racing at New York racetracks as a part of the prospective component of the study which covered season 2016 to 2018. Figure 3 shows the probability of continuing in racing/training as the number the age of the horse increases was declining. On the average the median age to experience BPSB was 4 years of age; which was similar to thage during 2010 – 2015. That means that up to 50% of the TB horses racing at NY racetracks injured with the 4 years of age. Among the TB horses, the incidence of CIs per start was 20%, while for MIs was it was 17% (the 95% confidence interval were 18-24% vs 12-22% respectively). Similarly, the average incidence of MIs during racing was 17% vs 29% during training. Although these differences make sense because only healthy horses that are perceived to be healthy by the trainers and confirmed by the veterinary staff at the pre-race exam are allowed to race. This could also be due to the fact that veterinarians only let healthy conditioned race, meanwhile there is no official control over the health condition of the horses while training fr future races. This finding also implies that the pre-race examination has a positive impact on the health of racehorses in general. We are planning to continue our analyses of additional collated factors, to discern the causal relationship of the putative risk factors.

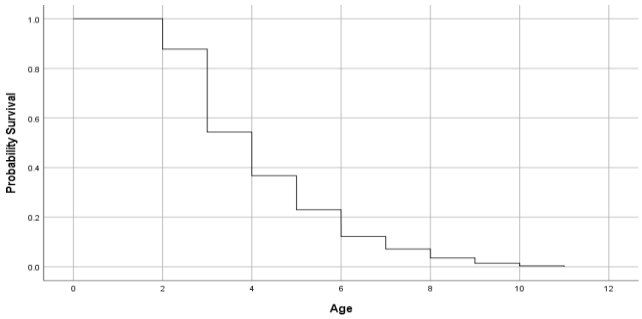


Figure 3. The incidence/probability of BPSB past certain number of life time starts

With regard to Aim 2, we have carried a couple of retrospective and prospective analytical epidemiologic studies. These studies were carried out using te matched case-control approach. For each BPSB fractured case two control horses were enrolled from the same race where the case was reported. The controls were chosen randomly. The purpose was to identify factors suspected to predispose to the risk of BPSB fracture. Several hypothesized risk factors were identified and analyzed (forty-three continuous (interval) and 17 categorical). We carried a systematic data analysis approach to shed light in the relationship between these factors and the risk of the BPSP fractures (Palmer et al, 2020). Factors found significantly associated with increased likelihood of BPSB fractures in the retrospective component included intact male horses, the number of high speed furlongs prior to the first start, age of the horse and the number of fetlock abnormalities recorded by regulatory veterinarians during the pre-race inspection on the day of the incident race (Palmer et al., 2020). Career weeks in training, racing as a 4-year-old, and age at the time of the first start were associated with a decreased risk for biaxial proximal sesamoid bone fracture. Figure 4 shows the relationship between the number of weeks in training and the risk of BPSB fracture. There was high risk of injury in the early training and this risk decrease overtime. Therefore, only horses who survive the early training would continue training. The implication is that most of the predisposing factors play role early in the career of the horse (Figure 4).

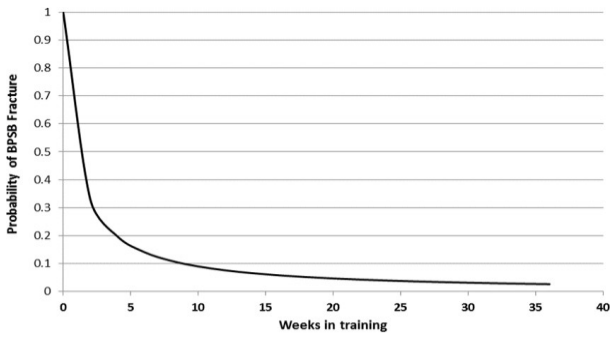


Figure 4. The relationship between the probability of BPSB fracture and the number of weeks in training

We also carried out a study to determine if race-day metacarpophalangeal pre-race inspection findings of Thoroughbred racehorses are associated with increased risk BPSB fracture. Using a similar approach of matched case-control study ninety racehorses that experienced a catastrophic BPSB fracture at NYRA racetracks and 180 control horses randomly selected from the same races as the case horses were enrolled in the study. The significance of association between the prerace inspection findings and the risk of BPSB fracture was assessed using conditional logistic regression analysis while considering the confounding factors. Metacarpophalangeal joint effusion and gait abnormality were significantly associated with increased risk for BPSB fracture. Horses with metacarpophalangeal joint effusion were 1.5 times as likely to experience BPSB fracture in comparison to horses without this finding. Similarly, horses with a gait abnormality (“wings,” “paddles” or “travels stiff, fair, choppy, rough or wide”) were at twice the risk for BPSB fracture. Additionally, the probability of BPSB fracture increased proportionately to the total number of metacarpophalangeal joint and gait abnormalities found during the pre-race inspection. Used in conjunction with other horse-specific risk factors, such as intact male horses and a high level of exercise intensity during early training, the finding of multiple

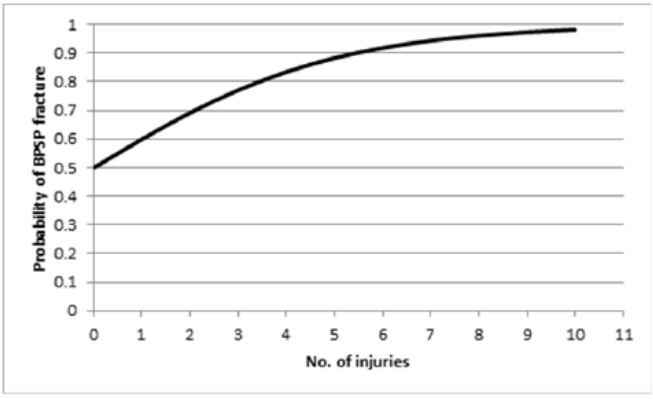


Figure 5. The relationship between the probability of BPSB fracture and the total number of fetlock abnormalities

metacarpophalangeal abnormalities, metacarpophalangeal joint effusion in 4 particular and a gait abnormality during the pre-race inspection can be helpful to identify Thoroughbred racehorses at increased risk for BPSB fracture. As an example of exposure to the interval factors that are associated with increased risk of BPSB fracture Figure 5 shows the relation between the total numbers of abnormalities detected at the pre-race examination and the risk of BPSB fracture. As the numbers of abnormalities increases the risk of fracture increases.

As a part of Aim 3, we carried out a couple of studies to standardize the enzyme immunosorbent assay (ELISA) for the detection of neurofilament (pNF-H) among control (non-injured) and injured horses (Intan-Shameha et al., 2017). The rational was to explore the use of pNF-H as a biomarker for detecting the possible neuronal in horses which could predisposes horses to MI or CE injuries. We found that pNF-H is an excellent biomarker for detecting neuronal death in horses. Figure 6 shows the risk of neurological disorder and the levels of pNF-H, as the levels of serum pNF-H increases the probability of neurological disorder increases (Intan-Shameha et al, 2017).

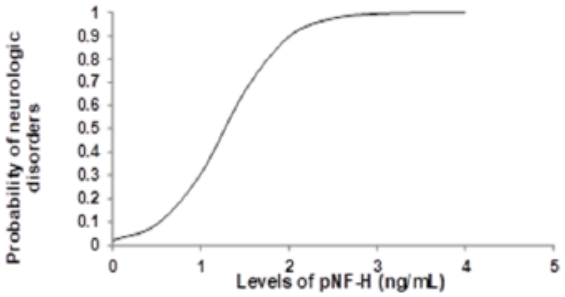


Figure 6. The relationship between the levels of neurofilaments (pNF-H) and the probability of Neurological disorders

We also assessed the association between the age of a control horse and the serum levels of pNF-H and found that there is a slight increase of pNF-H concentration with age (Morales-Gómez et al. 2019). The rationale was that we wanted to investigate whether the serum levels of pNF-H was affected with the age of the animal. The conclusion was that the relation between the serum levels of pNF-H and the age was not significant; hence the observed serum levels are mainly due to neuronal degenerations.

We have also been investigating the levels of the pNF-H among horses experiencing BPSB fracture and control horses. Preliminary indications showed that there might be a significant association between the injures and the pNF-H proteins (Figure 7). The interpretation would be that horses experienced BPSB fracture could have an underlining neuronal damage which might have predispose the horse to the injury. We want to forewarn the readers these are preliminary results and we will continue examining the levels of pNF-H levels among afflicted horses and controls and investigate this relation in the context of other predisposing factors.

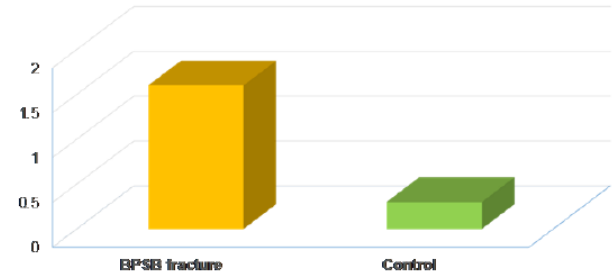


Figure 7. Concentration of pNF-H among the BPSB fracture cases and control horses

C. Significance

Emphasize the significance of the findings and their potential impact.

Through our studies we have identified some of the factors that predispose TB horses to the risk of racetrack injuries, namely BPSB fracture. These factors were shared with the trainers and the veterinary staff at the track as recommendation to modify their training regimen so that the risk of

BPSP fracture injuries to horses while racing would be lowered or eliminated. We are hopeful that the trainers will implement these recommendations in hope that the equine industry can maintain its livelihood and preserve the health of the racing horses. 5

We are continuing these studies to ascertain the roles of these factors and identify addition factors, if exist. The ongoing studies would also shed more light on the mechanism by which the identified factors exacerbate the risk and possible mitigation strategies.

As for the neurofilament study, we endorse the usage of the ELISA pNF-H as a potential biomarker tool to differentiate between horses that are experiencing neuronal degeneration from those that are not. Many veterinarians are beginning to use pNF-H concentration values, in addition to observed clinical signs, as an indicative sign of nervous system generation. As a result, we have had request from different veterinary clinics in the USA to help in this aspect. The finding on the levels pNF-H have broader clinical implication as a differential diagnostic tests in lameness.

D. Publications and Other Grant Submissions

If applicable, report publications resulting from the study, including manuscripts submitted or accepted for publication, and submissions and/or external grants resulting from the award.

Palmer S, Verderosa A, Morales-Gomez A, Mohammed HO. Pre-race inspection findings associated with biaxial proximal sesamoid bone fracture in Thoroughbred racehorses (2010-2018). J Equine Vet Science 2020; (Under review and revisions).

Palmer SE, Zhu S, McDonough SP, Mohammed HO. Risk factors associated with biaxial proximal sesamoid bone fractures in Thoroughbred racehorses at New York Racetracks 2010-2016. Equine Vet J 2020; (Under review and revisions).

Intan-Shameha AR, Divers TJ, Morrow JK, Graves A, Olsen E, Johnson AL, Mohammed HO. Phosphorylated neurofilament H (pNF-H) as a potential diagnostic marker for neurological disorders in horses. Res Vet Sci. 114:401-405. PMID: 28750210.

Morales Gómez AM, Zhu S, Palmer S, Olsen E, Ness SL, Divers TJ, Bischoff K, Mohammed HO. Analysis of neurofilament concentration in healthy adult horses and utility in the diagnosis of equine protozoal myeloencephalitis and equine motor neuron disease. Res Vet Sci. 2019; 125:1-6. PMID: 31103855.

Palmer SE, McDonough SP, Mohammed HO. Reduction of Thoroughbred racing fatalities at New York Racing Association racetracks using a multi-disciplinary mortality review process. J Vet Diagn Invest. 2017; 29(4):465-475. PMID: 28613116.

Morales Gomez AM, Palmer SE, Mohammed HO. Rate of attrition of Thoroughbred and Standardbred racehorses at New York Racetracks due to exercise related fatalities and injuries 2016 to 2018. Equine Vet Science. 2020 (In preparation).

Principal Investigator:	Dr. Gillian Perkins
Title:	Validation of an Equine Stall-side Major Crossmatch Test
Project Period:	12/1/17-5/31/20
Reporting Period:	1/1/19 – 12/31/19

The sampling of horses along with the crossmatch testing using both the reference test (LAB) and the stall-side kit (KIT) were completed. Aim 1 consisted of horses that were blood typed at the UC Davis Laboratory and were then crossmatched using both the LAB and KIT as expected compatible versus incompatible cross matches. For Aim 2, horses with unknown blood types were crossmatched by each test and agreement noted. The data was compiled, and the statistical analysis was performed in the past year. Dr. Fenn (Large Animal Medicine Resident) presented an abstract at the ACVIM Forum in June 2019 in Arizona. Then Dr. Fenn prepared the manuscript for publication which was then edited by the co-authors and has recently been submitted.

Principal Investigator:	Dr. Heidi Reesink
Title:	Intra-articular recombinant lubricin to restore joint lubrication and prevent osteoarthritis in horses
Project Period:	1/1/18 – 12/31/19
Reporting Period:	1/1/18 - 12/31/19

A. Specific Aims of the Study and Modifications

Specific Aims: If the aims have not been modified, state so. If they have been modified, provide the revised aims and the reason for the modification.

Aim 2 was modified due to unexpected results obtained in Aim 1, where IL-1□-induced synovitis resulted in increased synovial fluid lubricin concentrations. For this reason, Aim 2 was modified to assess similar parameters (synovial fluid inflammatory profiles, lubricating parameters, and lameness) in a joint lavage model, which was expected to result in "washout" of the synovial fluid lubricants lubricin and hyaluronic acid prior to evaluating treatment with intra-articular lubricin.

Aim 1. To investigate how IL-1□-induced synovitis affects equine synovial fluid inflammatory profiles, lubricating parameters and lameness.

Aim 2. To investigate how joint lavage affects equine synovial fluid inflammatory profiles, lubricating parameters and lameness.

B. Summary of Scientific Findings

Describe the studies directed toward the specific aims and the positive and negative results obtained. If applicable, address any changes to the innovative potential of the project. If technical problems were encountered in carrying out this project, describe how your approach was modified.

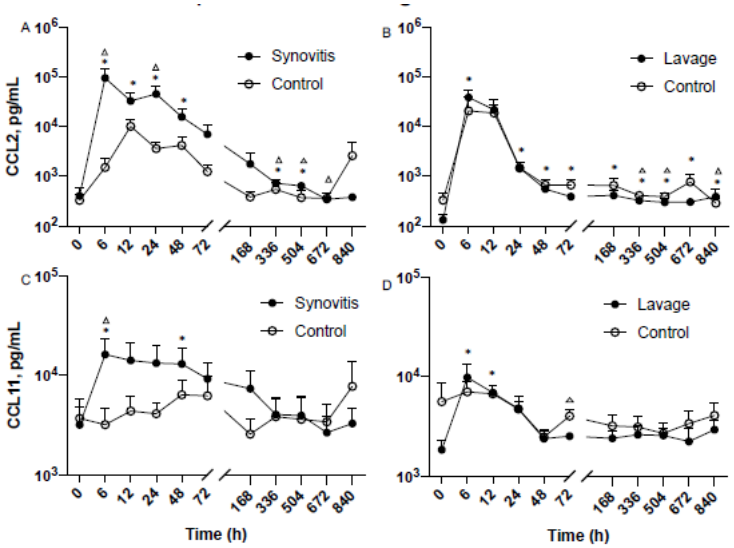


Fig. 1. CCL2 concentrations increased following synovitis (A) and lavage (B). The effect of repeated arthrocentesis is most apparent for the lavage model, where an acute but transient increase in CCL2 is observed (B). CCL11 concentrations increases following synovitis (C) and lavage (D). * = difference from baseline, Δ = difference from contralateral limb.

Six horses were utilized in a randomized block crossover study design. Middle carpal joint (MCJ) synovitis was induced with 100ng IL-1b, and the contralateral joint was injected with saline, followed by arthrocentesis at 0, 6, 12, 24, 48, 72, 168, 336, 504, 672, 840 hours. Following a 30-day washout period, intra-articular lavage with 2L lactated ringers solution was performed in one tarsocrural joint (TCJ), and both TCJ were sampled at the aforementioned timepoints. Clinical parameters were assessed, including lameness evaluation with an inertial sensorbased system. Synovial fluid biochemical parameters assessed included synovial fluid lubricants (lubricin, hyaluronic acid [HA]), inflammatory cytokines (prostaglandin E2, interleukin-1b, tumor necrosis factor a, CCL-2,-3,- 5,-11) and sulfated glycosaminoglycans (sGAG).

Microrheology was performed to assess synovial fluid viscosity. Synovial membrane biopsies were obtained at the end of each 35-day study period for histological analysis.

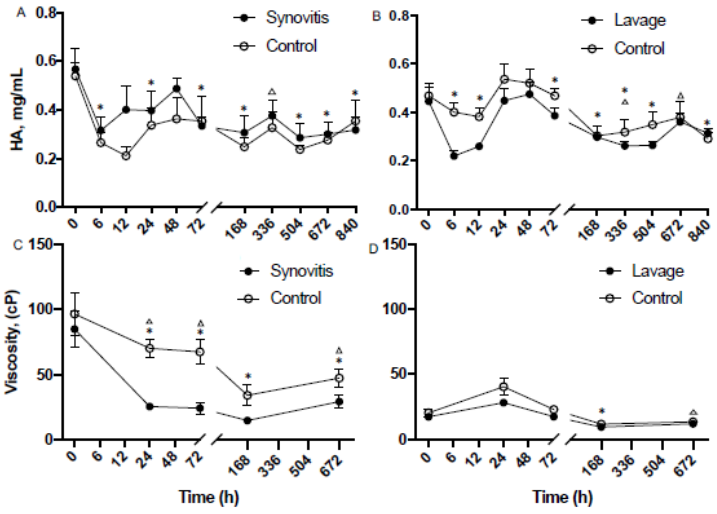


Fig. 2. HA concentrations decreased following synovitis (A) and lavage (B) and remained below baseline for the study duration. Viscosity decreased in both synovitis (C) and lavage (D), but to a greater extent in synovitis. * = difference from baseline, Δ = difference from contralateral limb.

For the purpose of obtaining the inference statistics, multilevel mixed-effects linear regression was used with specific groups as the fixed effects and confounded by the time of the sample, age and sex of the animal. The random effects were organized on three levels where the joint was nested within the specific leg and leg was nested within horse. All three levels were defined as random intercepts.

Synovitis resulted in a more severe inflammatory response based on traditional parameters (white blood cell, total protein, PGE_2, sGAG) as compared to lavage. Clinically, horses displayed a transient tachycardia, tachypnea, and elevated rectal temperature for the first 24 hours in the synovitis model, in addition to a significant but transient lameness. Lavage did not have an effect on these parameters. Synovial fluid white blood cell count and total protein increased for three days following synovitis and lavage; however, the magnitude of difference was greater in the synovitis model.

Interestingly, the chemokines CCL2 and CCL11 increased in concentration in response to both synovitis and lavage models with differences between affected and control joints only being seen acutely in the synovitis model (**Fig. 1**). All joints, regardless of treatment or model, demonstrated a decreased HA concentration, especially high molecular weight HA, and decreased viscosity. These changes were most dramatic when sampling was performed at more frequent intervals ≤ 24h, suggesting that repeated arthrocentesis may be the primary driving force behind the changes (**Fig. 2**). Contrary to our hypotheses, synovial fluid lubricin concentrations increased significantly during periods of frequent, repeated arthrocentesis for both synovitis and lavage models (**Fig. 3**).

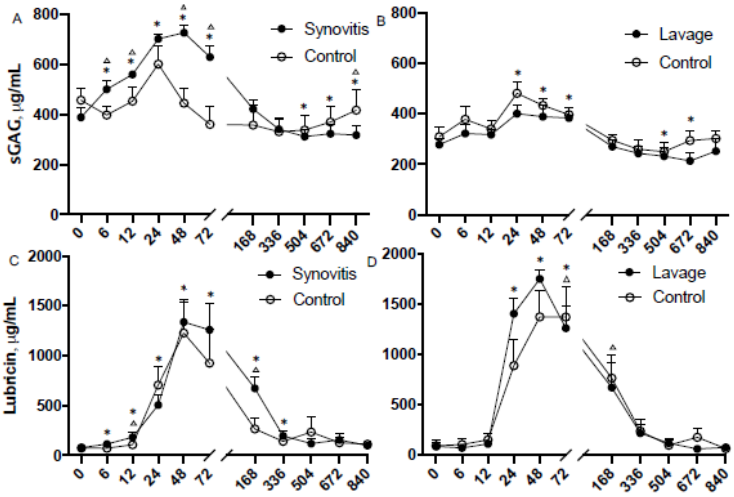


Fig. 3. sGAG concentrations increased to a greater extent following synovitis (A) than lavage (B), but also following repeated carpal arthrocentesis (A). Lubricin increased markedly and to a similar extent following synovitis (C) and lavage (D), suggesting that repeated arthrocentesis was the primary driving force for the increase in lubricin. * = difference from baseline, Δ = difference from contralateral limb.

Histological analysis was performed on the synovial membrane biopsies that were obtained at the end of each study period and were assessed using a synovitis grading scale that included: cellular

infiltration, vascularity, intimal hyperplasia, subintimal edema, and subintimal fibrosis. There were no significant differences appreciated between control and affected limb in either model, likely due to the extended duration of time obtained post-intervention (35 days).

C. Significance

Emphasize the significance of the findings and their potential impact.

Lameness is the most common reason for loss of use in the equine athlete, and the vast majority of lameness is attributed to osteoarthritis (OA). Options for treating OA are limited. Lubricin is a molecule that binds to cartilage and aids in lubrication of the joint via boundary lubrication—a type of lubrication that occurs under high-load, low-motion conditions. Lubricin supplementation has shown beneficial effects in several rodent models and, more recently, in a Yucatan mini-pig model; however, lubricin has not been evaluated in equine joint disease. The goal of this study was to select an appropriate equine model for the evaluation of lubricin therapy. Of significance, this study has shown that repeated arthrocentesis has a greater impact on lubricin and HA concentrations than the IL-1□ or lavage interventions, as demonstrated by the parallel changes in control and affected joints in both models. This information is critical for the design of future studies and suggests a potentially interesting biological response by lubricin to repeated arthrocentesis that warrants further investigation.

There are a limited number of non-terminal experimental models that can be used to study early inflammatory and biophysical changes that occur before radiographically evident OA in the equine joint. Comparing the IL- 1□-induced synovitis model, with its established inflammatory effects, to intra-articular lavage, a model we felt would significantly impact synovial fluid biophysical parameters, provides important information on model selection for future studies evaluating synovial fluid inflammation and joint lubrication. Many of the inflammatory markers evaluated in this study have not been previously investigated in equine synovial fluid, including CCL2, CCL3, CCL5, CCL11 and IL-1□. In collaboration with the Wagner lab, we have provided data about how this panel of synovial fluid inflammatory biomarkers changes in response to model interventions, which will be useful for other researchers and clinicians investigating intra-articular therapeutics in synovitis and lavage models. Chemokine receptor ligands (CCL) are molecules linked to inflammatory diseases, including periodontitis and rheumatoid arthritis. Specifically, these molecules are involved in the recruitment of inflammatory cells by bone and connective tissue. Chemokines have yet to be measured in an equine model.

The impact of this study is three-fold: (1) by determining how synovial fluid cytokines, lubricants and biophysical properties change over time in synovitis and lavage models, we provide important insight to guide selection of appropriate equine models for future experimental studies; (2) by elucidating chemokine concentrations over time, we have identified CCL2 and CCL11 as the most promising candidates for evaluating early inflammatory changes in equine synovial fluid in both experimental and clinical joint disease and; (3) by identifying dramatic increases in lubricin secondary to frequent, repeated arthrocentesis, we can advise against frequent (< 1 week) arthrocentesis in experimental studies investigating synovial fluid biophysical properties while motivating studies to determine what mechanisms are responsible for increased synovial fluid lubricin. Finally, these studies motivate the evaluation of synovial fluid, and possibly serum/plasma lubricin, as a potential biomarker for early joint disease in horses.

D. Publications and Other Grant Submissions

If applicable, report publications resulting from the study, including manuscripts submitted or accepted for publication, and submissions and/or external grants resulting from the award.

Publications

1. Watkins AW*, **Reesink HL.** Lubricin in experimental and naturally occurring osteoarthritis: A systematic review. Re-submitted, Osteoarthritis and Cartilage, Feb 2020.

2. Watkins A*, Fasanello D*, Stefanovski D, Schurer S*, Caracappa K*, D'Agostino A*, Costello E*, Freer H, Read C*, Noordwijk K*, Su J, Colville M, Paszek M, Wagner B and **Reesink H.** Comparison of synovial fluid lubricants and inflammatory cytokines following repeated arthrocentesis in equine synovitis and joint lavage models. In preparation for submission to: *BMC Veterinary Research*.

3. Watkins A*, Fasanello D*, Stefanovski D, Schurer S*, Caracappa K*, Noordwijk K*, D'Agostino A*, Costello E*, Freer H, Read C*, Su J, Colville M, Paszek M, Wagner B and **Reesink H.** Evaluation of intra-articular injections of non-animal, stabilized hyaluronic acid on synovial fluid lubricant and inflammatory cytokines in equine synovitis and joint lavage models. In preparation for submission to: *BMC Veterinary Research*.

Abstracts, Posters and Invited Talks

1. Watkins A*, D'Agostino A*, Caracappa K*, Schurer S*, Reesink H. Evaluation of a sustained release hyaluronic acid formulation in equine IL-1□-induced synovitis. Cornell University One Health Symposium, Ithaca, NY, Nov 17, 2018. Accepted for poster presentation.

2. Watkins A*, Fasanello D*, Stefanovski D, Schurer S*, Caracappa K*, D'Agostino A*, Costello E*, Freer H, Read C*, Noordwijk K*, Su J, Colville M, Paszek M, Wagner B and **Reesink H.** Comparison of synovial fluid lubricants and inflammatory cytokines following repeated arthrocentesis in equine synovitis and joint lavage models. In preparation for submission to the: *American College of Veterinary Surgeons* (ACVS) Annual Summit, Resident's Forum

3. Watkins A*, Fasanello D*, Stefanovski D, Schurer S*, Caracappa K*, Noordwijk K*, D'Agostino A*, Costello E*, Freer H, Read C*, Su J, Colville M, Paszek M, Wagner B and **Reesink H.** Evaluation of intra-articular injections of non-animal, stabilized hyaluronic acid on synovial fluid lubricant and inflammatory cytokines in equine synovitis and joint lavage models. In preparation for submission to the: 2020 *North American Veterinary Regenerative Medicine Association* (NAVRMA) Conference

Grant Proposals Submitted but Not Funded

A grant entitled "Equine-specific synthetic lubricin for joint therapy" was submitted to the Grayson Jockey Club Research Foundation for the 2019/2020 grant cycle; however, this grant was not funded. Therefore, our plan is to collect additional *in vitro* and *in vivo* data to bring this technology closer to clinical translation and re- submit this grant to the Grayson Jockey Club Research Foundation next year for the 2021/2022 grant cycle.

(Reesink, PI) 4/1/2019-03/31/2021
Grayson Jockey Club Research Foundation Equine-specific synthetic lubricin for joint therapy
The goal of this project is to design and synthesize equine-specific synthetic recombinant lubricin, assess its biological properties through transcriptomics-based approaches, and determine its residence time in healthy and inflamed equine joints.
Role: Principal Investigator

Grant Proposals Submitted and Funded

(Reesink, PI) 1/1/2020 - 12/31/2021
Zweig Memorial Fund
Unraveling Lubricin Signaling in Equine Joint Injury
The goal of this project is to identify signaling networks regulated by proteoglycan 4 expression and to determine how recombinant lubricin regulates cell and tissue properties in equine joints.
Role: Principal Investigator

(Reesink, PI) 4/1/2020 - 03/31/2022
Grayson Jockey Club Research Foundation Bisphosphonates and Fatal Musculoskeletal Injury
The goal of this project is to determine the prevalence of bisphosphonate use in racehorses and to determine whether bisphosphonates are associated with fatal musculoskeletal injury.
Role: Principal Investigator

Principal Investigator:	Dr. Heidi Reesink
Title:	Proximal sesamoid bone microdamage and fracture toughness in Thoroughbred racehorses.
Project Period:	6/1/18 - 5/31/20
Reporting Period:	1/1/19 – 12/31/19

The specific aims of this proposal are three-fold: 1) to determine if there is evidence of prodromal microdamage that occurs in PSBs prior to catastrophic breakdown, 2) to assess differences in fracture toughness between fractured and non-fractured PSBs using the unfractured limb (fractured contralateral limb – FXCL) for mechanical testing and 3) to correlate those findings with bone morphometries and densities. We hypothesized that sesamoid bones from horses sustaining PSB fracture would have more evidence of microdamage than controls. We also hypothesized that sesamoid bones from horses sustaining PSB fracture would have reduced fracture toughness in *in vitro* mechanical testing as compared to controls, which would result in decreased fracture tortuosity and decreased microdamage accumulation post-testing.

Techniques for evaluating bone microdamage include the use of radio-dense stains, which enable visualization of microcracks in bone in three dimensions via micro-CT imaging. Preliminary data has supported microdamage uptake by lead UA staining in PSBs **(Fig 1)**. All samples dedicated to lead UA staining have been stained and scanned with micro-CT. Image processing and interpretation is currently underway in Horos software.

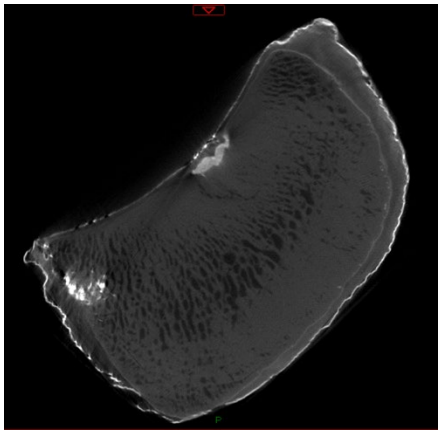


Figure 1. *Sagittal section of a control lead UA-stained PSB, revealing an area of increased lead UA uptake at the articular surface.*

Basic fuchsin is another technique for characterizing bone microdamage histologically. Designated samples have been bulk stained with basic fuchsin and embedded in plastic. Slides are being created for analysis. Preliminary analysis suggests that fluorescence imaging (excitation wavelength: 542-582 nm) is optimal for highlighting the presence of microdamage in our basic fuchsin-stained bone samples.

Forelimb proximal sesamoid bone morphometries (dimensions and volume) have been documented in 12 horses with fractured PSBs and 12 sex-age-matched-controls (SAMC). Table 1 outlines PSB descriptive statistics. There were no significant differences between PSB height, width, depth, and volume between horses with fractured PSBs and SAMC ($P \leq 0.05$).

Table 1. PSB Descriptive Statistics

	Height (mm)	Width (mm)	Depth (mm)	Volume (ml)
Fracture Horses	37.2 95%CI[36.6,37.8]	29.7 95%CI[29.3,31.1]	19.7 95%CI[19.4,20.1]	15.3 95%CI[14.9,15.8]
Control Horses	37.1 95%CI[36.3,37.5]	29.3 95%CI[28.9,29.6]	19.4 95%CI[19.1,19.7]	15.0 95%CI[14.7,15.4]

Microbeams were created from the PSBs in 12 fractured contralateral limbs (unfractured limb) and sex-age matched controls (SAMC) in preparation for fracture toughness testing. All beams have been tested in 3-point-bending to failure. Custom Matlab code is currently being optimized to analyze the data. Training and race records are currently being requested.

Principal Investigator:	Dr. Gerlinde Van De Walle
Title:	The Mesenchymal Stem Cell Secretome Against Equine Herpesvirus Type I Infections
Project Period:	1/1/18 – 12/31/20
Reporting Period:	1/1/19 – 12/31/19

Over the past year we have made significant progress on both aims of our proposal. For Aim 1; testing the efficacy of cell (MSC) secreted factors against Equine Herpesvirus Type I (EHV-1) *in vitro,* we carried out infection experiments using the three-dimensional nasal explant model we validated in year one of this study. We are currently analyzing the data from those experiments. Under Aim 2; testing the efficacy of MSC secreted factors against EHV-1 *in vivo,* we conducted a study to determine if MSC secreted factors inhibited EHV-1 in a well-established mouse infection model. We pre-treated mice intranasally with control medium or medium containing MSC secreted factors, then after 4 hours infected the mice intranasally with EHV-1 in control medium or medium containing MSC secreted factors. We weighed mice daily and collected lungs at day 2 post-infection to determine the severity of the infection. Although we detected no significant differences in mouse weight or infectious virus in the lungs, our histological analysis of lung tissues did indicate that there was more epithelial cell damage in the lungs of mice infected with EHV-1 in control medium compared to those mice infected with EHV-1 in medium with MSC secreted factors (Figure 1).

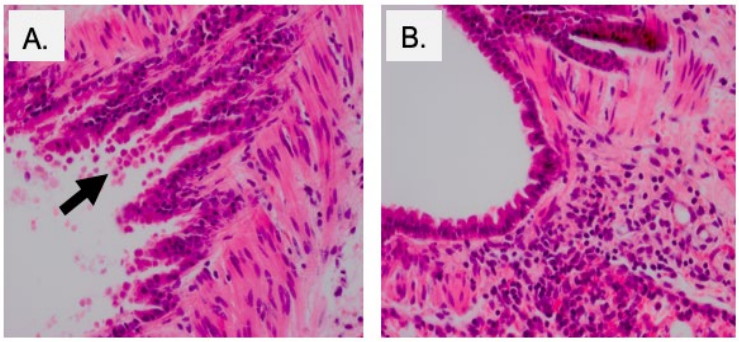


Figure 1. H&E images of mouse lungs collected at day 2 after EHV-1 infection in the presence or absence of MSC CM. ***(A).*** *Lung tissue from a mouse infected with EHV-1 in control medium. Arrow points to sloughed airway epithelial cells.* ***(B).*** *Lung tissue from a mouse infected with EHV-1 in the presence of MSC CM. Airway epithelium is healthy and intact.*

Principal Investigator:	Dr. Bettina Wagner
Title:	Intranasal Biomarkers of EHV-1 Susceptibility and Protection
Project Period:	1/1/19 – 12/31/20
Reporting Period:	1/1/19 – 12/31/19

The **specific aim** of this one-year project is to identify a comprehensive intranasal biomarker panel indicative for EHV-1 susceptibility and for host immunity and protection using a banked sample set from fully susceptible and fully protected horses.

The project uses RNAseq analysis of previously archived nasal secretion samples from horses that were protected against EHV-1 (n=4) or susceptible to infection (n=4). We are analyzing several samples from these horses pre-infection and at different time points post EHV-1 infection. The project goal is to identify novel biomarkers for protection against EHV-1 and those that point to susceptibility to the virus. The banked samples were reconstituted earlier this year and submitted for RNAseq analysis. Some of the samples required additional treatment because of the viral content and sampling at acute disease stages (sample quality decreased due to the active infection in some horses). Thus, these procedures and the sample sequencing took a little longer than originally assumed.

However, the sequencing results came back in mid-August, the initial analysis was performed at the RNAseq Core, and the outcome and data were sent to us early in September. We performed the initial data analysis and the data set looks really promising. At this point, five genes that show high differences between the EHV-1 susceptible and protected groups throughout the first two weeks post infection are evaluated by quantitative PCR in additional horses from the same challenge study. This step is done to confirm the up- or down-regulation of these genes before we are selecting the most robust genes as biomarkers of protection against or susceptibility to EHV-1. The initial RNAseq analysis also points to a variety of novel genes and biomarker candidates that can distinguish early and late infection stages between the two groups, infection stages within the susceptible group, and those that can serve as unique indicators of protection at different time points post EHV-1 exposure.

In addition to the proposed work, we have used parts of the unique nasal secretion sample set to perform EHV-1 PCR on protected horses. These results are also quite exciting. Remarkably, they are showing no indication of viral replication in protected horses and add additional confirmation to the hypothesis that immune horses do not shed virus when infected with EHV-1. This data piece further supports the hypothesis that protected horses are not posing any risk on outside horses when released earlier from quarantine.

Principal Investigator:	Dr. Bettina Wagner
Title:	Towards a neonatal vaccine against equine herpesvirus type 1 (EHV-1)
Project Period:	1/1/18 – 6/30/20
Reporting Period:	1/1/19 – 12/31/19

This project had two specific aims:

In **Aim 1**, we planned to further optimize the concept of neonatal vaccination with IL-4/EHV-1 antigen and to test if IL-4 is essential for neonatal EHV-1-specific memory B-cell activation. We vaccinated neonatal foals with IL-4/EHV-1 antigen to prime the immune system of the neonate for EHV-1 specific memory B-cell induction. We used four groups of foals: group 1 was vaccinated at birth with our neonatal IL-4/EHV-1 antigen vaccine; group 2 received EHV-1 antigen at birth (without IL-4), groups 3 and 4 were not vaccinated at birth. At 3 months of age, we vaccinated groups 1, 2 and 3 with a commercial EHV vaccine with the goal to boost EHV-1 specific B-cell development in the groups that previously received a neonatal vaccine. Foals typically mount poor antibody responses to commercial vaccines at 3 months of age. Group 4 was not vaccinated and served as control group.
We then tested protection provided by these vaccine combinations. All foals were weaned and experimentally infected at 7 months of age. EHV-1-specific antibody responses, including isotype responses, were evaluated in all groups at several times of the entire study. EHV-1 specific B-cells, T-cells and local cellular responses were also analyzed.
At this time, all experimental parts for Aim 1 are performed and all data are analyzed. We are working on the manuscript to publish the results.

The **major findings of Aim 1** are:

- Neonatal vaccination of foals with a simple EHV-1 antigen induces B-cell memory. We described this first after our initial neonatal EHV-1 vaccination approach (Wagner et al. 2017) and this project confirmed that this is a repeatable outcome.
- Neonatal vaccination also primes the immune system to respond earlier to conventional vaccines.
- Foals that are vaccinated at birth respond earlier and develop higher protective immunity after EHV-1 challenge.
- An increased immune response after EHV-1 challenge was also observed in foals that were not vaccinated at birth but received the conventional vaccine at 3 months of age. This response was however lower than the response of the neonatal vaccination group.

In **Aim 2**, we targeted the mechanistic evaluation of neonatal memory B-cell induction, expression profiles, and differences in those from adult horse memory B-cells. We are analyzing the influence of IL-4 on EHV-1 specific memory B-cell induction after neonatal vaccination, conventional vaccination and EHV-1 infection. A circulating EHV-1 specific B-cell population can typically be detected in the peripheral blood 4-5 days post after EHV-1 antigen stimulation. In the past year, we optimized our B-cell sorting protocol and can now reliably sort these cells from the frozen PBMC stocks that were stored while Aim 1 was performed and also from stored PBMC of vaccinated or EHV challenged adult horses. We are currently isolating the B-cells by cell sorting and activate the cells *in vitro* with our panel of EHV-1 antigens with and without IL-4 to analyze their antibody secretion. Aliquots of the sorted cells are set aside for RNAseq analysis. When all samples are sorted, these will be sent to the RNAseq Core and we will compare the RNA expression profiles in B-cells from the groups in Aim 1 and adult horses.

APPENDIX C
Summary of 2019 Expenditures

2019 Research Awards	$625,553
2020 Public Relations and Administrative Budget	$42,500
2020 Incentive Awards	$0
TOTAL EXPENDITURES:	**$668,053**

APPENDIX D
2019 Research Presentations

The 40th Anniversary Celebration of the Harry M. Zweig Fund for Equine Research at Cornell University's College of Veterinary Medicine was on November 13, 2019. The program is below.

Dean Lorin Warnick presents the Zweig family (L-R: Anne Zweig, Sylke Zweig, Brian Zweig) with a commemorative horseshoe mounted by Cornell's farrier.

Harry M. Zweig Memorial Fund for Equine Research

40th Anniversary Celebration

Wednesday, November 13, 2019

Schurman Hall, College of Veterinary Medicine, Cornell University

ORAL PRESENTATIONS 1:00-5:00 PM

moderated by Dr. Thomas Divers & Dr. Julia Felippe

Lecture Hall 5

1:00-1:15 PM	**Opening Remarks by Dr. Robert Weiss**
1:15-2:15 PM	**Practical Research Advances from Zweig Funding**
1:15-1:45 PM	Dr. Thomas Divers
1:45-2:00 PM	Dr. Norm Ducharme
2:00-2:15 PM	Dr. Gillian Perkins
2:15-2:30 PM	**Equine Program Overview**
	Dr. Lisa Fortier
2:30-3:00 PM	**Young Investigators, the Next Generation of Equine Researchers**
2:30-2:45 PM	Dr. Heidi Reesink
2:45-3:00 PM	Dr. Michelle Delco
3:00-3:15 PM	**Break**
3:15-3:45 PM	**Federal/Sponsored Funding Opportunities Resulting from Zweig Awards**
3:15-3:30 PM	Dr. Gerlinde Van de Walle
3:30-3:45 PM	Dr. Jonathan Cheetham
3:45-4:45 PM	**KEYNOTE: Building a Career in Translational Biomedical Science for the Benefit of Horses and Humans**
	Lauren Schnabel, DVM, PhD, DACVS, DACVSMR Associate Professor of Equine Orthopedic Surgery Department of Clinical Sciences North Carolina State University College of Veterinary Medicine
4:45-5:00 PM	**Closing Remarks by Dr. Robert Weiss**

POSTER SESSION & RECEPTION 5:00-6:15 PM

hors d'oeuvres, beer & wine

1st Floor Atrium

ZWEIG COMMITTEE & AWARD RECIPIENTS DINNER 6:30-8:30 PM

by invitation, RSVP required

2nd Floor Atrium

APPENDIX E
2020 Research Awards

CONTINUATIONS

Principal Investigator	Project Title	2020 Award
Antczak, Douglas	Functional gene annotation in the horse	$68,537
Cheetham, Jonathan	Accelerating recovery after Clinical Sciences Laryngeal Nerve Graft in Horses	$99,643
Reesink, Heidi	Does Proximal Sesamoid Bone Mineral Loss Lead to Increased Fracture Risk?	$83,921
	SUBTOTAL:	**$252,101**

NEW AWARDS

Principal Investigator	Project Title	2020 Award
Antczak, Douglas	2020 Horse Genome Project Workshop at Cornell	$7,000
Delco, Michelle	The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease	$55,768
Felippe, Julia	Diagnostic markers in mares with placentitis	$78,244
Kelly, Kathleen	Genomics of Autopsy– Negative Sudden Cardiac Death in Racing Thoroughbreds	$76,782
Reesink, Heidi	Unraveling lubricin signaling in equine joint injury	$57,621
Van de Walle, Gerlinde	Studying the replication kinetics of equine parvovirus hepatitis (EqPV-H)	$49,552
Wagner, Bettina	Nasal immunity and its function in preventing transmission of EHV-1 in immune horses	$71,571
	SUBTOTAL:	**$396,538**

APPENDIX F
Zweig News Capsules

Issue #67 (June 2019) and #68 (December 2019) are attached.

The full archive (1988 – present) is available online at https://ecommons.cornell.edu/handle/1813/22528.

Zweig

From the Harry M. Zweig Memorial Fund for Equine Research at Cornell University College of Veterinary Medicine

No. 67 August 2019

Dr. Heidi Reesink named next Harry M. Zweig Assistant Professor in Equine Health

By Patricia Walddron

Heidi Reesink has been named the Harry M. Zweig Assistant Professor in Equine Health in honor of her ambitious research program to detect horses at risk for catastrophic injuries and to develop new treatments for arthritis.

The professorship is a three-year, endowed position for a junior faculty member who shows great promise for advancing equine research. It can be instrumental in helping junior faculty secure funding and develop high-level publications necessary for long-term success. Reesink has received grants previously from the Zweig Memorial Fund to support individual research projects. She has also received support from the Grayson-Jockey Club Research Foundation, the Cornell Center for Advanced Technology, the Cornell Center for Materials Research and the National Institutes of Health through a Mentored Clinical Scientist Development Award, a highly competitive grant to advance the careers of promising researchers.

"Dr. Reesink is recognized as a rising star among junior faculty and an important contributor to our college community," says Robert Weiss, Associate Dean for Research and Graduate Education. "One of the main concerns of the Harry M. Zweig Memorial Fund is catastrophic racehorse injury. Solving this problem is a critical need in the racing industry and she's doing some exciting work in that area."

Catastrophic musculoskeletal injuries – mainly broken legs – are the main cause of death for racehorses. "We would like to understand how these fractures occur and to develop better methods to screen for racehorses at risk of fracture," says Reesink.

She is working with Dr. Scott Palmer, the equine medical director for the New York State Gaming Commission, in addition to epidemiologists and pathologists to examine horses that died after sustaining fractures to their proximal sesamoid bones (PSBs) – two knobby, triangular bones at the back of the fetlock joint. Often, racehorses with this injury have no telltale signs of lameness during pre-race examinations or X-rays. Reesink is comparing the PSBs from the uninjured leg of those horses to PSBs from horses that died of other causes, using advanced CT scans. She hopes to develop better screening procedures to identify horses susceptible to fractures.

Heidi Reesink, Ph.D. '16

Reesink is also looking into new treatments and early detection methods for arthritis. "Joint disease and osteoarthritis are the leading cause of lameness in horses, but there are limited options for treating arthritis in horses and in humans," says Reesink. "A long term goal is to develop better therapies, that will both provide longer and better pain relief and that, ideally, will prevent or delay the development of arthritis."

After an injury, many – but not all – horses develop arthritis, so Reesink is examining the synovial fluid that bathes the joints to identify biomarkers that would indicate which horses are at risk and might benefit from preventive therapies. She is also investigating lubricin, a sugar-coated protein in the synovial fluid that provides lubrication, to see if an injection of lubricin can treat lameness. Additionally, along with pharmaceutical industry colleagues, Reesink is testing whether horses benefit from human arthritis medicines that are not available on the veterinary market.

Reesink is passionate about "one medicine," the concept that human and veterinary biomedical research can each inform the other. She believes that while providing treatment for animals, she can also offer insights to advance human health, and hopes her work will translate into clinical applications that benefit both horses and people.

As a former athlete herself, Reesink has fractured bones and injured joints while playing volleyball, competing in tae kwon do events, snowboarding and running, and so she understands the challenges and potential for developing better treatments for sports injuries. "I saw equine orthopedic surgery as a way to combine my love of the horse as well as my desire to advance the science of sports medicine."

Powers combined: Cornell D.V.M. - Ph.D.'s unite skills to advance veterinary medicine

By Olivia Hall

Veterinarians who also hold a Ph.D. are a rare breed, but two programs at the College of Veterinary Medicine are working to increase their number.

Students may arrive with a D.V.M. in hand and earn a Ph.D. in Biomedical and Biological Sciences (BBS), or they launch directly into the Combined D.V.M.-Ph.D. Degree (CD) Program. Whether completed consecutively or together, both graduate degrees contribute distinct perspectives to shape researchers uniquely positioned to tackle cutting-edge issues in veterinary science.

"A veterinary degree teaches you a lot about how to recognize, diagnose, and treat disease, but not a lot at all about how those things were found out," said John Parker Ph.D. '99, associate professor of virology with the Baker Institute for Animal Health and the Department of Microbiology and Immunology. "If you want to be able to advance the field, you need advanced training in a discipline that's going to allow you to do that."

At the same time, veterinarians' broad education in animal physiology and health problems prepares them to identify important issues across species and conduct impactful translational research. "The students tend to be more analytical how these problems arise, what's known about their causes and how they're being treated," said Dr. Hélène Marquis, professor in the Department of Microbiology and program director and oversight committee chair of the CD program. "They make connections that others without the veterinary medicine knowledge would not necessarily make as easily."

But the path to becoming a D.V.M-Ph.D. can be long and expensive, so that only few choose to pursue it.

"Securing a position in the Graduate Training Program in Comparative Medicine for Veterinary Scientists (GTPCM) is what gave me the confidence to take that leap," said Michelle Delco '98, D.V.M. '02, Ph.D. '16, assistant research professor in the Department of Clinical Sciences. She began her career as a dedicated equine surgeon but became frustrated with diagnosing injuries that carried poor prognoses and had no good treatment options. "I came back to academia because I wanted to help change that," she said.

Delco completed her Ph.D. with three years of support from the GTPCM, which has been backed by a grant from the National Institutes of Health since 1990. (Other D.V.M.s seeking a Ph.D. at Cornell may receive state-supported graduate research assistantships as an alternative source of funding.)

While students who fill the three or four available GTPCM positions every year cover a wide range of interests, quite a few – like Delco – have worked in equine surgery and medicine, according to Parker, the program's director. "That's because it's almost impossible to get a job as a faculty member in equine surgery without a Ph.D.," he explained. The program allows them to spend up to ten percent of their time on clinical work to maintain their surgical skills while getting advanced training.

Meanwhile, the Combined D.V.M.-Ph.D. Degree Program - developed in 2001 out of an existing Veterinary Scientist Training Program – accepts two to three students annually. After they complete both degrees over the span of about seven to eight years, it forgives their D.V.M. tuition loans.

"The students are extremely self-motivated, successful in winning fellowships, and proactive in terms of what they want to do with their degrees," Marquis said. Like individuals admitted to the GTPCM, they have been selected for the depth of their research experience, as well as their likelihood to thrive in an intense program and contribute to veterinary science.

Cornell in turn offers a very strong research community and a lot of flexibility. "Students can find a research lab at the CVM or down campus, as long as that person has a connection to BBS," Marquis said.

In addition, Parker makes sure trainers participating in the GTPCM have a record of successful mentorship and external funding that can pay for students' research experiments. "It wouldn't be surprising that a graduate student would cost $24,000 in supplies per year," he said. "Science is expensive."

This careful approach to selecting both students and trainers has proven successful: graduates have advanced into a variety of positions in academia, government, and pharmaceutical and biotechnology industries. Delco, for one, has been very happy with her choice to go through the GTPCM. "Anyone considering research as part of their career should learn more about this program," she said. ■

Michelle Delco'98, D.V.M. '02, Ph.D. '16

Cornell looks to spur expansion in equine programs

By Melanie Greaver Cordova

The college's equine programs may soon be expanding their impact in the local community thanks to a new proposal crafted by a committee at the Cornell University College of Veterinary Medicine (CVM). "The committee wanted to assess where we are now and where we're going," said committee chair Lisa Fortier, Ph.D. '98, the James Law Professor of Large Animal Surgery.

In April of 2018, Lorin D. Warnick, D.V.M., Ph.D. '94, the Austin O. Hooey Dean of Veterinary Medicine, tasked the Equine Programs Planning Committee to recommend steps for the college to maintain excellence in equine education, research and clinical service. Warnick instructed the committee to consider how the college can best remain the number-one choice for students, clients and faculty.

Fortier approached this tall order with systematic precision: Gather a committee of well-informed staff and faculty; outline all of the college's equine programs and their reach; gather extensive community feedback; and create a comprehensive report that could be shared broadly with the CVM community. "It was a very healthy process," said Fortier. "A lot of people came to the meetings to share what they thought about what we were already doing well, and where they see us going in the future. We got very good feedback."

Dreaming big

Some plans for the equine programs are already underway, such as transforming the Cornell Equine Park into a working farm. "It helps the veterinary students to see a working farm, where you do your own hay and manure, rather than a university model where you hire all those tasks out," Fortier said.

The park may become more versatile in other ways, too. "One of the visions we have for the park is that it becomes something like the Lab of Ornithology," she said. "While we're designing the new barn, do we design it only for horses, or do we have an entertainment space, a space where people can get married and hold events."

She and her team have already started hosting events there, like "Hoof it for the Horses," a 5K race raising money to send Cornell Equine-focused veterinary students to the American Association of Equine Practitioners meeting. "We started the race last fall, and that helped to send nine veterinary students to the conference. It was a huge success."

The Cornell Equine Park's Sarah Ruby leads one of their warmblood horses.

A sturdy foundation

The new report on equine programs, submitted to the dean in early 2019, offered administrative and programmatic recommendations. Their top three suggestions were ordered in terms of priority and impact:

(1) An equine primary care and farrier hire, who would work in the field with all Cornell-owned animals as well as Cornell's referral base. "Everything in equine programs comes back to having the best clinicians in the world," said Fortier. "If you don't have the best clinicians, if you don't attract the best residents or students, then you don't attract the caseload — it's very cyclical."

(2) Retain the Cornell Equine Park and proceed with consolidation of a new facility, which would include redesigning the park to maximize its role as a recruitment and teaching tool.

(3) Review the current mechanism of admission and funding for graduate students with an interest in equine research, specifically those seeking a dual degree.

In addition to the top three recommendations, the report further outlined suggestions for Cornell Ruffian Equine Specialists, hospital caseload, the overall veterinary curriculum, the equine summer school course and presence at the New York State Fairgrounds. Fortier anticipates many of these recommendations being implemented over the coming months, further strengthening the college's focus on education, research and clinical care. ■

Dr. Thomas Divers, clinican and clinical investigator

By Lauren Cahoon Roberts

One might call him horse healer — others might call him horse sleuth — either way, Dr. Thomas Divers, the Steffen Professor of Veterinary Medicine at the Cornell University College of Veterinary Medicine (CVM), has saved the lives of countless horses worldwide thanks to his drive to solve puzzling clinical cases.

"My passion has been treating clinical cases," says Divers. "Therefore, my research has always been clinically driven. I'll see a disease in clinical practice and recognize that the disease needs further investigation." Divers notes that the research he's done during his almost 30-year tenure at Cornell has always been collaborative; "I have never solved any clinical mystery by myself." Thanks to Divers and his research collaborators, some major equine diseases are no longer the deadly threats they once were.

Vitamin fix

One of Divers' favorite projects while at Cornell was on Equine Motor Neuron Disease (EMND). This frequently fatal degenerative condition affects motor neurons and results in muscular wasting and weakness and remains the only naturally-occurring animal disease model that mimics Lou Gehrig's disease in humans.

EMND was a mystery up until the nineties, when Divers, along with renowned CVM faculty Dr. Hussni Mohammed; Harold "Skip" Hintz, M.S. '61, Ph.D. '64; John Cummings '58, D.V.M. '62, Ph.D.'66; and Alexander de Lahunta, D.V.M. '58 Ph. D. '63, discovered that it was an oxidative disorder caused by vitamin E deficiency. "One of the highlights of my career was traveling with Mohammed and Cummings to a great many equine stables throughout the Northeast United States, Brazil, Ireland and Switzerland to investigate field cases of EMND," says Divers. "I can still hear Dr. Cummings emphasizing how important it was to visit every case possible, regardless of where it was located, in order to better understand the epidemiology and to let practitioners and horse owners know that we were trying our best to determine the cause."

Doing so, however, amounted to many 15-plus-hour days driving round-trip around the Northeast in all sorts of weather to examine affected animals, collect samples, fill out risk factor surveys and, most importantly, speak with the owners and attending veterinarians.

Dr. Thomas Divers. Photo provided

Unfortunately, Cummings passed away just prior to the research group's experimental reproduction of the disease which confirmed that prolonged vitamin E deficiency was the cause of the disease. "Cummings was perhaps the most brilliant individual that I worked with in my career and also one of the most modest," says Divers. Today, thanks to their research efforts, horses rarely suffer or die of EMND due to owners and feed manufacturers now providing adequate supplies of vitamin E.

The search for the suspect

The bulk of Divers' research has focused on infectious diseases, including another favorite Cornell-based investigation that searched for the cause of acute equine hepatitis, a frequently deadly condition also known as Theiler's disease or serum hepatitis. The disease, first reported in South Africa in 1919, was most commonly associated with recently administered equine blood, serum or plasma, but an etiologic cause had not been found.

Divers and his long-time research collaborator and close friend, Dr. Bud Tennant, James Law Professor of Comparative Medicine Emeritus, had been searching unsuccessfully for 30 years for the cause of Theiler's disease. They had theorized the disease was viral, but could not find proof for this hypothesis.

In 2011, an outbreak of the disease in horses on a Nevada farm reignited their focus on the disease. "We got really serious about our investigation because of both the

Nevada outbreak and recent advances in deep sequence technology that could lead to identification of previously unknown infectious agents," says Divers. Tennant had collaborated with hepatitis C researchers at Novartis, and asked them to investigate the Nevada samples for a similar virus. From that investigation, they found a novel RNA virus which they named "Theiler's disease associated virus" (TDAV). In Zweig-funded research, Divers and Tennant next explored the association of this virus with other North American field cases of Theiler's disease. With TDAV as their suspect, Divers and Tennant gathered and tested samples from horses with acute hepatitis from around the country, but TDAV was not present in the other diseased horses. Furthermore, later experiments performed at Cornell confirmed that the virus (TDAV) did not cause liver disease.

Although disappointed, Divers and Tennant broadened their search for a causative agent. They next collaborated with Columbia University and Rockefeller University to conduct a broader-based deep sequencing on a sample from a Nebraska horse that had died from Theiler's disease, and the tetanus antitoxin that had been administered to the horse.

This time, they found a previously undiscovered parvovirus. This DNA virus was named Equine Parvovirus — Hepatitis (EqPV-H) — and when two research horses at Cornell were inoculated with this newly discovered parvovirus, the horses developed acute hepatitis.

Armed with this knowledge, Divers and Tennant collaborated with Dr. Edward Dubovi's molecular virology laboratory in the Department of Population Medicine and Diagnostic Sciences. The team found the new virus in 18 consecutive equine cases of serum hepatitis, in the blood products the horses had received four to ten weeks earlier, and in the same Nevada horses that had ignited the investigation to begin with.

Thanks to these findings, the USDA Center for Veterinary Biologics now requires that all equine blood plasma and serum commercial products are tested and proven free of equine parvovirus, a policy move that should save the lives of numerous horses. Sadly, Tennant passed away in November of 2016, but studies on the immunopathology and transmission of the disease continue at Cornell with Divers and the viral hepatitis research team, which includes Dr. Gerlinde Van de Walle, associate professor, post-doc Joy Tomlinson, D.V.M. '10, and graduate student Mason Jager, D.V.M.'12..

Divers has led several other clinical investigations on equine hepatic diseases at Cornell, including determining the mechanism of biliary obstruction in horses with right displacement of the left colon; discovering that fall panicum (Panicum dichotomiflorum) hay caused several outbreaks of acute equine liver failure in the Mid-Atlantic area; and, along with Irish Equine Centre colleague Dr. Ursula Fogarty, discovered that red fescue grass caused liver disease outbreaks in horses in France.

Divers and colleagues also provided the first report of multiple adult horses developing acute hyperammonemic encephalopathy secondary to enteric disease, a condition that is now a commonly observed clinical syndrome.

Bacterial infections – puzzles, progress and problems

Divers has also investigated, with fellow CVM faculty member Dr. Yung-Fu Chang, professor in the Department of Population Medicine and Diagnostic Sciences, equine Lyme (borreliosis) disease and leptospirosis. Chang and Divers have performed experimental infection, antibiotic treatment and vaccine efficacy studies on equine Lyme disease — all supported by Zweig funding. "More research is needed," says Divers. "There are still many questions to which we do not have answers, including the percent of infected horses that have Lyme disease, and the full spectrum of clinical signs. One of the biggest frustrations of my research efforts has been the lack of help we have provided to practitioners on Lyme disease — I wish we could have done more!"

Divers then noted that research can veer in unintended directions sometimes. "As we were studying doxycycline and minocycline treatments for equine Lyme disease, practitioners reported to me that many stiff and lame horses had responded clinically to these antibiotic treatments — yet testing revealed that they had not been exposed to B. burgdorferi." Divers relayed this information to Dr. Lisa Fortier, Ph.D. '98, James Law Professor of Large Animal Surgery, whose lab confirmed the anti-inflammatory effects of doxycycline and minocycline on equine synovial membranes and cartilages.

Divers has also worked closely with Dr. Chang's laboratory to unravel some of the mysteries around equine leptospirosis, a bacterial disease that can cause uveitis in adult horses and abortions in mares. In Kentucky, leptospirosis is considered one of the most common infectious cause of abortions in mares and is

therefore of great concern for the equine industry there. Divers and Dr. Nita Irby, Ruttenberg Senior Lecturer of Ophthalmology and Divers' spouse, believed that equine recurrent uveitis (ERU) is often associated with Leptospira infection, and recent publications now suggest the condition is associated with 50 percent of ERU cases, possibly higher in warmblood horses. Divers and Irby visited Fort Dodge Veterinary Biologic Company 13 years ago to encourage the development of an equine Leptospira vaccine. In 2015, the company (now Zoetis) released a USDA approved equine vaccine, with Divers and Chang performing the preliminary Leptospira experimental infection model studies. The vaccine is now widely and successfully used in endemic areas to prevent abortions.

With retirement on the horizon for Divers, he said he's happy to pass his investigative torch to "the next generation of clinical researchers who are better trained than myself," says Divers. "I hope these younger researchers enjoy clinical research as much as I have, and develop collaborations with other researchers and equine veterinarians that are both fun and successful in improving the health of horses." ■

Dr. Thomas Divers. Photo provided

Thank you to former Zweig Committee Vice-Chair Robert Williams

Robert Williams

We bid a fond farewell to Robert Williams, who stepped down from his position as vice-chairman of the Zweig Committee in 2018, following his appointment and confirmation as Deputy Secretary for Gaming in 2017. Williams served on the Committee for five years. Williams is also chairman of the New York State Franchise Oversight Board, representing the interests and overseeing financial operations of the State's racing franchise. Prior to his appointment, Williams served as executive director of the New York State Gaming Commission, and brought crucial insight to the Committee on the views and needs of New York's racing industry.

Williams has held numerous positions in the industry, including assistant counsel for the New York State Racing and Wagering Board, acting director of the New York Lottery, special assistant counsel in the Executive Chamber, counsel to the New York State Task Force on Casino Gambling, and executive director of the New York State Committee on the Future of Racing.

We are grateful for his leadership and dedication to the Zweig Committee.

2019 HARRY M. ZWEIG MEMORIAL FUND FOR EQUINE RESEARCH AWARDS

The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state's equine racing industry.

In 1979, by amendment to the pari-mutuel revenue laws, the New York State Legislature created the fund to promote equine research at the College of Veterinary Medicine, Cornell University.

New Awards

$72,954 to Dr. Douglas Antczak for "Functional Gene Annotation in the Horse"

$98,385 to Dr. Jonathan Cheetham for "Accelerating Recovery after Laryngeal Nerve Graft in Horses"

$57,540 to Dr. Michelle Delco for "The Role of Mitochondrial Damage Associated Molecular Patterns (mDAMPs) in Equine Joint Injury and Disease"

$61,351 to Dr. Heidi Reesink for "Does Proximal Sesamoid Bone Mineral Loss Lead to Increased Fracture Risk?"

$72,568 to Dr. Bettina Wagner for "Intranasal Biomarkers of EHV-1 Susceptibility and Protection"

Continuations

$88,254 to Dr. Heidi Reesink for "Intra-articular Recombinant Lubricin to Restore Joint Lubrication and Prevent Osteoarthritis in Horses"

$74,578 to Dr. Gerlinde Van de Walle for "The Mesenchymal Stem Cell Secretome against Equine Herpesvirus Type I Infections"

$99,923 to Dr. Bettina Wagner for "Towards a Neonatal Vaccine against Equine Herpesvirus Type 1 (EHV-1)"

www.vet.cornell.edu/public/research/zweig

40th Anniversary of the Zweig Fund

With support from the Harry M. Zweig Memorial Fund for Equine Research, the College of Veterinary Medicine has been able to conduct cutting edge research benefiting the equine species while helping to ensure a healthy and positive future for the horse racing industry.

Cornell has developed equine research projects in the following areas supported, in part, by the Zweig Fund: reproduction, orthopedics, genetics, cardio-respiratory function, nutrition, and infectious diseases. The Zweig Fund has also been instrumental in supporting the careers of young equine researchers through the Harry M. Zweig Assistant Professorship in Equine Health and the Zweig Equine Clinical Fellowship program.

To mark the 40th anniversary of the Zweig Fund, the College will be hosting a series of presentations, followed by a poster session and reception to share its accomplishments with the broader equine community. The event is held in appreciation of the Zweig Committee's support and to promote a greater awareness of equine health and research.

The presentations are scheduled for November 13, 2019, at the College of Veterinary Medicine, Cornell University, Ithaca, New York, beginning at 1:00pm.

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2019 HARRY M. ZWEIG MEMORIAL FUND COMMITTEE

The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers and veterinarians.

Scott Ahlschwede, D.V.M.
Rood & Riddle Equine Hospital
Saratoga Springs, NY

Chad Brown
Saratoga Springs, NY

Gabriel Cook, D.V.M.
New England Equine Practice
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Janet Durso, D.V.M.
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M. Kelly Young
Agriculture & NYS Horse Breeding Development Fund
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Brian Zweig
Rensselaer, NY

Cornell University
College of Veterinary Medicine

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S3-216 Schurman Hall
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Zweig Memorial Trot 2019

Vernon Downs, Vernon, NY | Saturday, August 17th | Post time 6:10 PM

Information: 1-877-888-3766 | vernondowns.com/racing

Visit us online
vet.cornell.edu/zweig

Our site provides information on the projects and publications resulting from the Zweig Memorial Fund, and demonstrates the objectives of the Fund in promoting equine health in the racing industry. The Zweig News Capsule is published twice a year, and can be downloaded at bit.ly/ZweigNews. Please encourage other equine enthusiasts to visit the site.

Zweig

From the Harry M. Zweig Memorial Fund for Equine Research at Cornell University College of Veterinary Medicine

No. 68 December 2019

Zweig Fund celebrates 40th anniversary

By Lauren Cahoon Roberts

The Harry M. Zweig Memorial Fund for Equine Research celebrated its 40th anniversary Nov. 13 at the Cornell University College of Veterinary Medicine with poster presentations, research talks, and toasts and celebrations among researchers, students and committee members.

Zweig's tangible impact on equine health was on display during presentations from both long-time Zweig-funded equine researchers and the next generation of investigators.

"Since its inception, the Zweig Fund has distributed almost $17 million to investigators at the College of Veterinary Medicine, and this research support has been really critical to establishing Cornell as a leader in equine research," said Dr. Robert Weiss, associate dean for research and graduate education, in his opening remarks. "You'll see how the Zweig Fund supports important discoveries and some of the practical applications of those discoveries."

Dr. Thomas Divers, the Rudolph J. and Katharine L. Steffen Professor of Veterinary Medicine, introduced and moderated the first set of talks, which covered the practical research advances that Zweig funding has helped. He noted that Zweig has aided fundamental science findings, such as the first complete description of the equine genome, as well as insights into neonatal immunology and equine Lyme disease.

Divers also showcased many direct clinical applications, including the Cornell Collar and standing tie-back surgeries for treating recurrent laryngeal neuropathy;

Dean Lorin Warnick with Anna, Sylke and Brian Zweig

Jerry Bilinksi '66, D.V.M. '69 (center), was honored at the anniversary dinner

stem cells for preventing equine arthritis; and vitamin D supplements to prevent equine motor neuron disease. Zweig funding has helped bring about the equine Lyme antibody test used by practitioners all over North America, as well as the new leptospirosis vaccine now heavily used in the thoroughbred industry.

Divers also explained how Zweig funding has saved equine lives. New York racetracks have seen a 48 percent reduction of equine racing fatalities in the past five years thanks in part to Zweig-supported research on leading risk factors. Zweig research has also helped unravel the mystery of Theiler's disease and its association with equine parvovirus. Because of Cornell's Zweig-funded work, the USDA now requires that all horses used as blood product donors must be negative for equine parvovirus. Finally, equine practitioners now commonly administer heparin after abdominal surgery to help prevent adhesions — a discovery funded by Zweig.

Following Divers' overview, Drs. Norm Ducharme and Gillian Perkins gave summaries of their research efforts in upper airway problems and equine herpesvirus, respectively, pointing out how Zweig funding has been instrumental in pushing their advances forward.

After the review of past successes at Cornell, the presentations shifted toward future plans.

Lisa Fortier, Ph.D. '98, the James Law Professor of Large Animal Surgery, introduced the upcoming goals for all of Cornell's equine programs, outlining the plan to build their caseload, develop an equine field service and build a new horse barn.

Newer investigators Heidi Reesink, Ph.D. '16, and Michelle Delco '98, D.V.M. '02, Ph.D. '16, gave overviews of their equine research. Reesink discussed her studies on osteoarthritis and fractures with a focus on ending the epidemic of racehorse breakdowns, while Delco discussed mitochondrial dysfunction's role in early joint injury.

Next, Dr. Gerlinde Van de Walle and Jonathan Cheetham, Ph.D. '08, gave overviews of Zweig-funded work that had garnered federal/sponsored funding; Van de Walle highlighted her work in stem cells and viruses, while Cheetham discussed his research on peripheral nerve injury and recurrent laryngeal nerve disease.

Next, Lauren Schnabel, D.V.M. '04, Ph.D. '13, associate professor at North Carolina State University, gave her keynote talk, discussing her interest in using mesenchymal stem cell and platelet-rich plasma as therapeutics for a wide range of equine issues.

At the close of the presentations, Weiss noted, "I think

Dr. Thomas Divers and Lisa Fortier, Ph.D. '98

it's clear that the Zweig fund has had a transformative impact on equine research and has also fostered the creation of a remarkable community of equine scientists. Thanks to our partnership with Zweig, we are positioned to continue to make great strides in equine health."

Attendees then had the chance to mingle over hors d'ouevres and examine posters showcasing the latest Zweig-funded research.

Later, Zweig committee members, members of the Zweig family and other guests gathered in the second floor of the atrium for a celebratory dinner. During the dinner, Lorin D. Warnick, D.V.M., Ph.D.'94, the Austin O. Hooey Dean of Veterinary Medicine, presented Anna, Brian and Sylke Zweig with a mounted horseshoe from the Cornell farrier shop as a symbol of appreciation for Harry Zweig's impact on Cornell research. Warnick also publicly recognized Jerry Bilinksi '66, D.V.M. '69, a dedicated equine practitioner, who served as the New York State Senate representative to the Cornell University Board of Trustees. Warnick presented Bilinski with a mounted and inscribed brick from Schurman Hall.

As the event wound to a close with scientists, students and equine industry leaders sharing stories and insights, it was clear that the legacy of Harry Zweig has become a powerful engine for discovery and service for horses in New York and beyond. ■

The 2019 Zweig Memorial Fund Committee

One of several lectures during the annual Zweig meeting this fall at the Cornell College of Veterinary Medicine

Cornell scientists uncover unusual genetic diversity in Norse horse

By Lauren Cahoon Roberts

When the Vikings brought a few hardy horses to a remote Arctic island free of pests and pathogens, leaving them alone for a thousand years to breed, evolve and endure natural disasters — the resulting genetic diversity in today's herds was the opposite of what scientists at the Cornell University College of Veterinary Medicine had predicted.

"We found a highly unexpected level of heterozygosity and genetic variation in the Icelandic horse breed," says Dr. Doug Antczak '69, the Dorothy Havemeyer McConville Professor of Equine Medicine in the veterinary college's Baker Institute for Animal Health. "This is not the norm in many other horse breeds."

In a study published in the journal Genes & Immunity, researchers in the Antczak lab discovered this remarkable level of genetic diversity in a specific genetic region — that of the major histocompatibility complex (MHC), a set of genes coding for cell-surface proteins that help identify foreign molecules. These genes are ubiquitous in vertebrates and play a key role in adaptive immunity.

Antczak has devoted a good portion of his career to studying MHC. "I was just fascinated by it," he says. "When I began studying this for my Ph.D., very little was understood about it at that time." Fast forward to current day, and much is now understood about MHC — including the full genomic structure and polymorphism of the equine MHC region. As a result, scientists have the ability to test the makeup of an individual horse's MHC genes, including what copies of MHC genes a horse received from its parents. Many horse breeds, such as the Thoroughbred, have relatively little MHC genetic diversity, as a result of over 100 years of intense selective breeding by horse breeders.

The Icelandic horse, meanwhile, could not be more different from the Thoroughbred. This tough, multicolored equine breed evolved on the remote island of its namesake, descended from Viking steeds that arrived in the year 1000, with no further introductions of new horses since that time. The breed has endured several population crashes due to natural disasters, which often causes extreme declines in genetic diversity due to the drop in numbers.

To top it off, Icelandic horses also evolved untouched by most major equine pathogens and biting flies — all

elements that are thought to shape and shift the MHC complex to mount adaptive immune responses to such foreign assaults — and, in theory, drive greater MHC diversity as some animals developed immunity advantages over others.

Given the breed's unique set of evolutionary circumstances, Antczak and his research team, then-undergraduate research fellow Camille Holmes and graduate student Nathaniel Violette, D.V.M. '18, wanted to examine MHC diversity of the Icelandic horse.

To do so, the group obtained samples from the Ithaca-based herd of Icelandic horses belonging to their CVM colleague, Dr. Bettina Wagner, chair of the Department of Population Medicine and Diagnostic Sciences, as well as Icelandic horse biobank samples from University of Iceland colleague Dr. Vilhjálmur Svansson. Each herd comprised related individuals: a stallion, several mares and their offspring. Using blood samples from each of the total 156 horses, the team identified and categorized each horse's MHC geneotype.

Their results were surprising; all but one of the horses was heterozygous for MHC, meaning they had mismatched pairs of the MHC genes. Additionally, their MHC samples yielded 79 unique haplotypes (a set of DNA variations that are inherited together), which is a remarkable level of diversity for one gene group.

"The full extent of MHC haplotype variation in the entire Icelandic horse population is unknown, but conceivably could number in the hundreds," says Antczak. "This was unexpected. It has led us to believe there is a genetic shuffling mechanism in place to drive this diversity."

That mechanism, they pose, involves the exchange of genetic information between chromosomes, forming fresh new combinations — known as 'recombination' in geneticist speak. Antczak and other horse geneticists believe the MHC may be a recombination hot spot in the horse genome, keeping those genes fresh even in the face of diversity-diminishing circumstances such as natural disasters or remote islands.

"This discovery illuminates how integral MHC diversity is to a species' survival," says Antczak. "In the case of Icelandic horses, the animals have evolved a mechanism to ensure that these genes continue to remix and refresh." ■

This and opposite page: Icelandic horses, a breed which has retained remarkable genetic diversity despite enduring several population crashes due to natural disasters.

Dr. Elaine Claffey joins Cornell Ruffian Equine Specialists team

By Olivia Hall

Elaine Claffey is the newest member of the Cornell Ruffian Equine Specialist (CRES) team. Recently certified as a Diplomate of the American College of Veterinary Surgeons (Large Animal), the graduate of the Virginia-Maryland College of Veterinary Medicine is involved in all aspects of care at the clinic, from elective and emergency surgery to sports and regenerative medicine and lameness work.

"Coming from the equine hospital in Ithaca, I knew I'd be joining a fantastic team of specialists here at CRES," said Claffey, who spent a year interning at the Vermont Large Animal Clinic before completing a residency in large animal surgery and continuing on as a clinical instructor at Cornell. "This clinic has such a unique place in the Long Island community, and I'm excited to be a part of that."

Her close relationship with horses informs how she approaches her work. "It definitely gives me a valuable outlook when working as an equine veterinarian," Claffey said. "It's easy for me to put myself in place of my clients when they are stressed or worried about their horses, because I'm the same way about mine. It also often helps me sort out subtle lamenesses or performance problems because I have that perspective as a rider."

Wrangler, an 11-year-old quarter horse gelding, was one of Claffey's more memorable patients. The show-winning horse had been hiding a painful condition known as "kissing spine," a condition in which the vertebrae touch or grind against each other. Claffey saw Wrangler for an orthopedic exam to look for any lameness or pain after Wrangler had been bolting and acting up with his owner, Anjanette Nicolazzo. "These cases of behavioral problems or poor performance can be tricky to sort out; fortunately at Cornell we have the strength of multiple different specialty disciplines so we can work together to solve the problem from many different angles," said Claffey.

After ordering X-rays of Wrangler's spine, Claffey discovered that 11 of Wrangler's vertebrae suffered from kissing spine; four were so severe they had fused together. It was one of the worst cases Claffey had ever seen.

Claffey performed the complicated, four-hour surgery with colleagues Dr. Jackie Hill and Dr. Norm Ducharme. Using X-ray guidance, the team cut parts of the vertebrae that were touching to provide more space for comfortable movement. After a yearlong recovery period, Wrangler was back under saddle and performing once again.

Claffey will continue to help patients and owners in her new role at CRES. "Dr. Claffey brings enthusiasm, dedication and incredible work ethic to Ruffian," said Dr. Norm Ducharme, chief medical officer at CRES. "She is one of the finest and brightest surgical residents graduating from the Cornell program, and in addition to her competence she has personal qualities that will help support the core value of our clinic: she is kind and compassionate. We are fortunate to have her." ■

Dr. Elaine Claffey

Cornell Ruffian Equine Specialists in Elmont, New York

Leroy Coggins inducted into hall of fame

By Patricia Waldron

Leroy Coggins, Ph.D. '62, an internationally-recognized virologist, former researcher at the Baker Institute for Animal Health and inventor of the "Coggins test" for equine infectious anemia (EIA), was recently inducted into the Harness Racing Museum's Immortal Hall of Fame in Goshen, New York.

Originally from North Carolina, Coggins earned a veterinary degree from Oklahoma State University in 1957 and then completed his Ph.D. at Baker in 1962, back when it was called the Veterinary Virus Research Institute. He studied viruses that cause diarrhea with Baker's founding director and eventual namesake, James Andrew Baker, Ph.D. '38, D.V.M. '40. Later, Coggins returned to Cornell and developed his famous test for EIA, which has been instrumental in controlling this deadly and untreatable disease in horses.

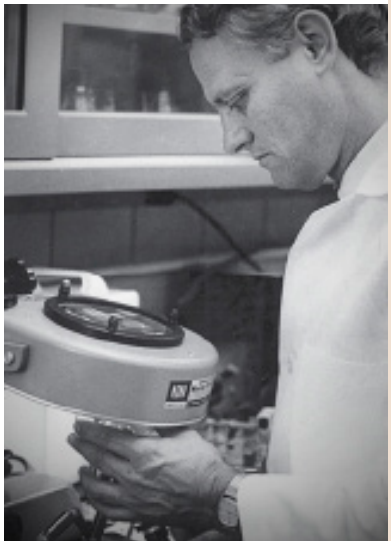
"Dr. Coggins was a soft-spoken gentleman with a southern drawl. He was thoughtful and considerate, and very dedicated to advancing veterinary medicine through research on virus diseases," said Dr. Douglas Antczak '69, the Dorothy Havemeyer McConville Professor of Equine Medicine, who met Coggins shortly after joining the Baker faculty.

The virus that causes EIA spreads through biting flies and an infected horse can expose others for a long time before showing symptoms. There is no vaccine or cure for the disease, making early detection vital to controlling its spread. Approved in 1973, the Coggins test is now required in many states when transporting a horse across state lines or for participation in horse shows and racing.

"The incidence of EIA is so low in the U.S. now that most veterinarians have never seen a case," said Antczak. "The disease is not yet eradicated in the U.S., but may be someday."

In 1980, Coggins returned to his home state as a founding department chair in the newly established North Carolina State University College of Veterinary Medicine program. He passed away in 2014.

A plaque commemorating Coggins' contributions to equine veterinary medicine will be hung at CVM. ■



Leroy Coggins, Ph.D. '62

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