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Shedding New Light on Laminitis

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SHEDDING NEW LIGHT ON LAMINITIS

Penn Vet continues to piece together this complex disease

BY SALLY SILVERMAN

Laminitis — commonly known as “founder” — is a painful and life-threatening disease in which the tissue bonding the coffin bone of the foot and the hoof wall becomes inflamed. And it is a disease that has confounded horse owners and veterinarians for ages.

But it wasn't until the untimely loss of legendary Thoroughbred Barbaro, the 2005 Kentucky Derby winner and hands-down favorite to earn Triple Crown status that year, that this painful disease drew international attention.

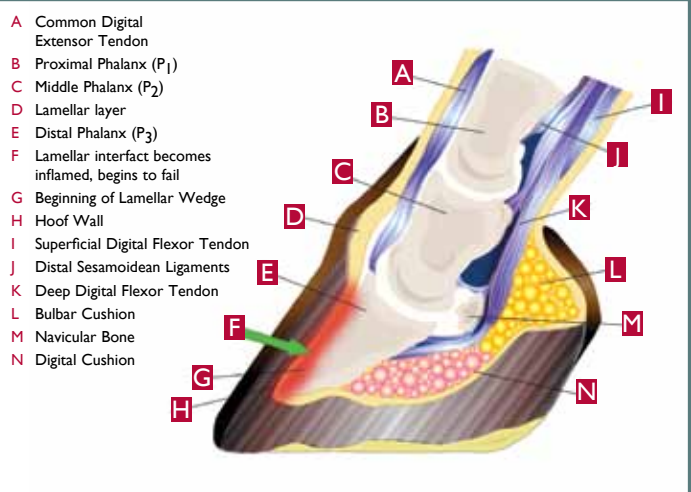
Since then, Penn Vet has been one of the leaders in laminitis research and discovery, and, in 2008 initiated the Laminitis Institute. In the past five years, Penn Vet's leadership role has expanded to involve a collaborative, multi-disciplinary approach from the molecular level to the mechanical; from the understanding of the potential causes of laminitis and its prevention; to treating its clinical signs and minimizing its painful effects.

While there remain mysteries surrounding this complex disease, Penn Vet researchers, working collaboratively with equine researchers worldwide, have made significant strides to better understand laminitis.

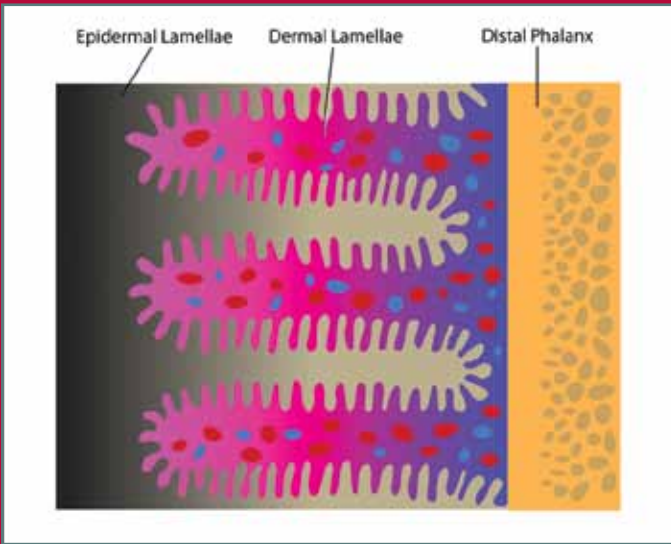
Here, we'll take a look at defining the disease as well as the projects currently underway, which continue to shed light on laminitis and give hope to horse owners and veterinarians, as well as novel treatment methods for consideration.

LAMINITIS: WHAT IS IT, EXACTLY?

Afflicting approximately 15 percent of horses in the United States in their lifetime*, laminitis is an inflammation in the tissue that connects the inner wall of the hoof with the distal phalanx of a horse's foot. Composed of an epidermal layer at the hoof wall, and a dermal layer associated with the bone, the laminae suspend the horse's axial skeleton (See illustration, above right). When the laminae become



An anatomical cross-section of a hoof with acute laminitis.



This graphic illustrates the interface of the epidermal and dermal lamellae with the distal phalanx.

Friends of Barbaro

From the moment Barbaro stole the international spotlight with his valor, athleticism and grace, he also stole our hearts. Thank you to the Friends of Barbaro who shared your resources with Penn Vet in honor and in memory of this brilliant Thoroughbred. On this anniversary we salute you for your support in helping to continue his legacy and to further our mission to find a cure for laminitis.

Joan C. Hendricks, V'79, GR'80,
The Gilbert S. Kahn Dean of Veterinary Medicine

inflamed and separate, the stretching of the laminar nerves and increased pressure on the soft tissue of the horse's foot cause severe pain and lameness. (See illustrations below and to the right.)

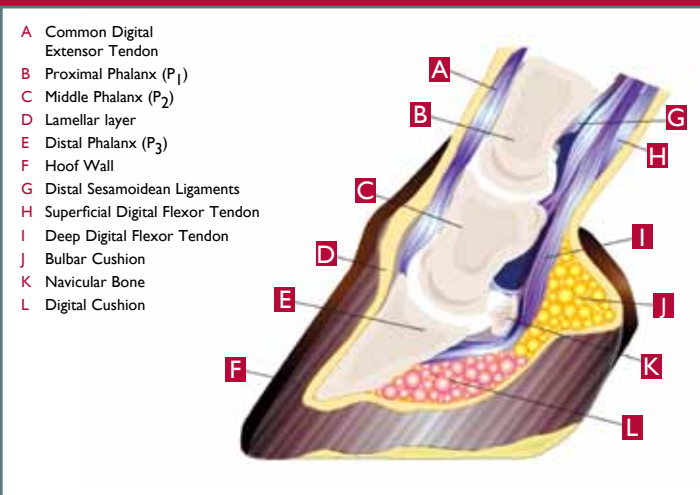
UNDERSTANDING THE DISEASE

Laminitis is typically secondary to another medical situation. It can be the result of metabolic syndrome or obesity, which are associated with impaired insulin function and share some similarities with pre-diabetes in people. It can also occur when an injury to one limb makes the horse favor another limb, putting added pressure on that foot. Horses with colic, pneumonia or a uterine infection are at much higher risk of developing laminitis.

Another mystery that remains surrounding the disease is its progression – something that Dr. Mary Robinson, lecturer in the Department of Clinical Studies at New Bolton Center, is studying by looking at the nitrous oxide signaling pathway and the response of blood vessels

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An anatomical cross-section of a healthy hoof.

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Dr. Hannah Galantino-Homer serves as lead investigator of the Laminitis Research Institute.

to inflammation. Dr. Robinson is interested in learning whether inflammation or lamellar pathology comes first. That is, does laminitis cause the inflammatory response or is the inflammatory response a symptom of the disease?

An acute episode of laminitis is associated with increased digital pulses, heat in the hoof and pain, all of which are suggestive of inflammatory disease. Activation of immune cells, especially macrophages, results in increased nitric oxide production, which is hypothesized to contribute to the pathophysiology of laminitis.

Dr. Robinson is also examining serum proteomics methodologies that, it is hoped, will aid in the identification of biomarkers of the developmental phase of laminitis. Though this stage is clinically silent, it is believed that this is the ideal time for intervention.

“There is nobody else in the world looking at this,” said Dr. Hannah Galantino-Homer, senior research investigator for the Laminitis Research Initiative at New Bolton Center. “This novel area of research could prove to have important implications with regard to etiopathogenesis, progression or treatment of laminitic disease in horses.”

Because the disease is rife with unanswered questions, it was clear that collaboration with other leading researchers in the field was essential.

And so, in order to better understand this disease and coordinate international research efforts, the Laminitis Institute at New Bolton Center, under the direction of board-certified surgeon Dr. James Orsini, associate professor of surgery, was created.

The goal of coordinating collaborative research has been attained. Since the Institute’s inception, there has been a marked increase in the numbers of scientific publications on the topic, featuring authors from more than one institution, as well as increased numbers of funded projects with co-investigators from multiple institutions.

“Many colleagues at Penn, University of Georgia, the Ohio State University, Texas A&M University and

University of Queensland, to name a few, have been working hand-in-hand to advance our understanding and management of laminitis,” said Dr. Orsini.

LAMINITIS RESEARCH INITIATIVE: THREE MAJOR PROJECTS

Headed by Dr. Galantino-Homer, the Laminitis Research Initiative at New Bolton Center has three major ongoing projects:

1. The Laminitis Discovery Database
2. The Grayson-Jockey Club Research Foundation Project
3. Stem Cell Studies

“My hopes for these three projects are that the Laminitis Discovery Database will provide us and others with the samples and information needed to conduct collaborative research; that the Grayson project will increase our understanding of the disease process and disease biomarkers; and that the stem cell studies will provide a better understanding of normal and diseased lamellar biology as well as a laboratory model for testing hypotheses at the cellular and molecular level,” said Dr. Galantino-Homer.

The Laminitis Discovery Database

The Laminitis Discovery Database, established in 2008 and maintained through a grant from the Bernice Barbour Foundation, has been one of the most valuable tools for facilitating collaboration between scientists.

Most previously published research on laminitis uses experimental models, with very little investigation involving naturally occurring disease. But this database has the potential to change that.

The Laminitis Discovery Database banks tissue and serum samples collected from horses with naturally occurring laminitis and a control group of horses not afflicted by the disease that have died or been humanely euthanized for other reasons.

To date, there are approximately 40 sets of samples from each group, all processed in multiple ways for a variety of uses. Blood and tissues are banked for molecular studies and diagnostics; photos are taken along with a history and description of the horse’s body condition; and lamellar tissue and bone slices are sent to Penn Vet’s Pathology Lab at New Bolton Center.

Information obtained from these samples is compiled for equine disease research and is available worldwide. The Laminitis Discovery Database is a valuable provider of real-life samples and information not previously available, but necessary for Penn Vet and other researchers to conduct collaborative research to further the study of equine disease and laminitis.

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Dr. Julie Engiles, assistant professor of pathology at Penn Vet, is one of the researchers on the New Bolton Center campus utilizing the database samples. Dr. Engiles uses micro-computer tomography technology to study the effects of laminitis on bone density and osteolysis, a prominent feature of laminitis that has not been previously investigated. The project is aimed at correlating laminitis-associated pathology that occurs at both the macro and micro-anatomic levels with pathology that occurs on the molecular level. She is also using the database to establish a scoring system for laminitis histopathology.

By using the samples, New Bolton Center board-certified pathologist, Dr. Engiles, explained, "We have developed a very detailed histologic grading scheme, which specifically characterizes the micro-anatomic changes that differ between clinically normal horses and horses with laminitis."

The Grayson-Jockey Club Research Foundation Project

Dr. Galantino-Homer's collaborative Grayson-Jockey Club Research Foundation Project (began in 2008 with the Laminitis Institute's Drs. Orsini and Christopher Pollitt, Dr. Rebecca Carter and Dr. Neal Rubinstein of Penn Medical Center, as well as Dr. Bhanu Chowdhary, an expert in equine genomics of Texas A&M University) is a second ongoing project, which investigates the gene and protein expression profiles of two models of laminitis—the oligofructose model meant to mimic some forms of pasture laminitis caused by excess oligofructans, a type of sugar, and the hyperinsulinemia model intended to provide a model for endocrinopathic laminitis which is associated with insulin resistance and high circulating insulin levels.

"If we can identify proteins that are being produced or degraded during the early stages of laminitis, they might be used as biomarkers for the identification of at-risk horses or targets for therapeutic intervention," said Dr. Galantino-Homer. "Since 2009, the availability of the horse genome sequence makes it possible to do a lot more than we could before."

The proteomic studies are currently in the data analysis stage and should be presented this year.

According to Dr. Galantino-Homer, they provide insight into the proteins that are degraded in the laminitic foot, contributing to the biomechanical failure and loss of digital support. In addition, these studies reveal differences between insulin-related and oligofructose-induced laminitis in proteins that mediate inflammation. They have also provided quantitative data on the protein composition of the laminae, key to understanding the changes in keratin expression profile changes with laminitis, which, in turn, affect the biomechanics of the foot.

Stem Cell Studies

Dr. Galantino-Homer's third project area is involved with the study of equine basal epithelial cells from the laminae and, in collaboration with Dr. Makoto Senoo, an epithelial stem cell biologist at Penn Vet's Department of Animal Biology and Institute for Regenerative Medicine, is in the process of developing an *in vitro* culture system for the study of equine laminitis.

"Stem cell studies will provide a better understanding of normal and diseased lamellar biology and a laboratory model for testing hypotheses at the cellular and molecular level," explained Dr. Galantino-Homer.

The project offers researchers a way to see what is happening to the cells at "ground zero" of laminitis: the epidermal basal cells that normally anchor the epidermal laminae to the dermal laminae.

Side-by-side evaluation, for example, of pharmaceutical agents for the treatment of laminitis, and investigation of trigger factors on isolated lamellar epidermal cells will also become a possibility. The epithelial stem cell study is also exciting for its potential as a therapeutic method: using stem cells for tissue regeneration in the laminitic hoof, much as it has been used in regenerative medicine for burn victims and corneal scarring.

RELATED RESEARCH

While the Laminitis Research Initiative is aggressively pursuing three projects that will directly impact the laminitic horse, there is other research happening at New Bolton Center that, while not being developed exclusively for laminitis, may be applicable to the disease.

One area includes pain management, a huge concern for horse owners and veterinarians when treating a horse that has foundered.

Dr. Kirsten Wegner, a board-certified anesthesiologist in the Section of Anesthesia and Critical Care at New Bolton Center, is working to refine an objective, quantifiable pain scoring system similar to that used in pediatric medicine. The clinical pain scoring system includes information such as the horse's position in the stall, degree of alertness, whether standing or recumbent, frequency of weight shifting from limb to limb, and most importantly, how the horse responds to a caregiver prompting them to walk or lift a hoof.

By recording each observation and interaction, the horse's behavioral responses and physiologic parameters, such as heart and respiratory rate, clinicians will have the ability to discern subtle changes in comfort level early on, and make appropriate changes in pain management strategy.

Working towards this goal, Dr. Wegner is developing an acute nociceptive (pain-causing stimulus) testing

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device for use in freely moving horses, which may be adaptable to a laminitic horse.

IN THE MEANTIME...

While the research component of the Laminitis Institute as well as independent research outside of the realm of specifically targeting laminitis is shedding new light everyday, horses continue to founder and, as a result, novel prevention, treatment and pain management techniques are also being discovered.

In 1916, a report from the USDA noted that the treatment of laminitis is probably more varied than of any other disease.

“Almost a hundred years later,” said Patrick Reilly, chief of Farrier Services at New Bolton Center, “we are faced with the same dilemma. There is a wide range of mechanical treatments for the laminitic hoof, but little scientific evidence to support any methodology.”

Cryotherapy

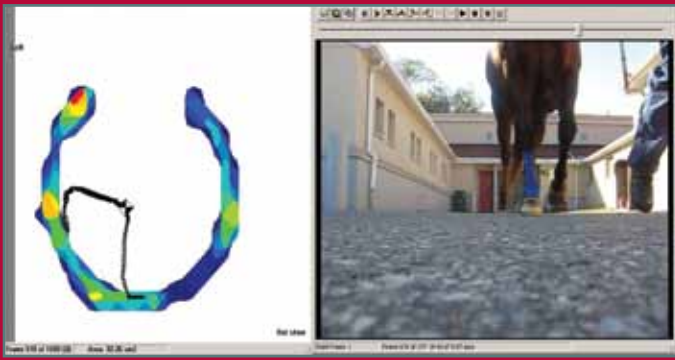
One potential way to prevent laminitis involves cryotherapy. It is hypothesized that cryotherapy, cooling the inflamed foot, would be effective in preventing a laminitic episode, but in the past such therapy has been limited to unwieldy methods such as hauling buckets of ice to the horse. It is labor-intensive and doesn't last.

Through a collaborative effort, Drs. Orsini and Andrew van Eps, now at University of Queensland, have developed a chilling system that circulates icy cold water through a boot that envelops the hoof wall and pastern. It provides a dry cold, in high volume, at a very low temperature.

“It seems like cooling the vulnerable limb or limbs two to four days beyond the systemic condition can prevent a laminitic episode,” said Dr. Orsini. “We are looking to our new Equi-Assist home nursing care program (see page 20) to



The mobile in-shoe force measuring system sensor mat in place under a glue-on shoe.



The synchronization of a video of a horse in motion (right) and the resulting force on the hoof (left). The force on the inside of the hoof (red) and the outside of the hoof (green) are plotted over several strides.

provide data that will help us to identify those horses who may be at risk for a laminitic episode before it begins.”

Improving shoeing to support the laminitic hoof

In addition to Dr. Orsini’s preventative cooling method, Reilly is working to develop an accurate in-shoe force measuring system to quantify the effects of the various treatment approaches relating to laminitis. In-shoe force measurements, with innovations developed at New Bolton Center, are proving valuable in quantifying the effectiveness of shoeing systems. The mobile in-shoe force measuring system sensor mat is cut to the shape of the foot and placed between hoof and horseshoe using the glue-on method of shoeing developed at NBC.

“Given the hundreds of years of anecdotal mechanical treatments,” said Reilly, “my hope is that measuring the effect of various shoeing techniques might enable us to better understand and improve our ability to support the laminitic hoof.”

Current areas of study include the effect of hoof capsule integrity, which is compromised during laminitis, and the effect of the shoe positioning in aiding the rehabilitation of the laminitic horse.

FORWARD MOMENTUM

While much has been accomplished to better understand this debilitating disease, researchers at Penn Vet and beyond know there are still strides to make.

“As we focus on the challenges in front of us, it is important to recognize the efforts of the many trailblazers, researchers, owners, caregivers and colleagues who continually advance the understanding and treatment of laminitis through their dedicated work,” said Dr. Orsini. “We stand on the shoulders of their efforts for even greater future accomplishments with our vision to conquer laminitis.”

*Moore RM. *Vision 20/20 – Conquering equine laminitis by 2020. Proceedings. 2nd AAEP Foundation Equine Laminitis Research Workshop, pp 11-15.*

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