Mary Anne McPhail Dressage Chair: Stephanie Valberg DVM PhD DACVIM, ACVSMR

Research associates:
Keri Gardner BS MS, Laboratory Manager
Sudeep Perumbakkam BSc MS PhD, Genomics and Bioinformatics

Veterinary students: Zoe Williams BSc, Kaitlin Maier BSc

Undergraduate students: Melissa Schott, Marissa Henry, Megan Bertels, Lexi Kraniak
Mission

Owners report that 25% of dressage horses experience muscle pain and muscle weakness that impairs the athletic elegance, expressive animation, strength and suppleness key to success in dressage and sport horses. Horses experiencing muscle pain show signs of stiffness, sore backs, unwillingness to work, and vague hindlimb lameness and the exact cause often goes unrecognized. We aim to improve the performance of dressage horses through our overarching goal of defining the basis for neuromuscular disorders in horses, developing accurate, minimally invasive diagnostic tests, and producing optimal methods for prevention or management of these conditions. Through enhancing muscle coordination, strength and health we can improve the lives and performance of dressage horses.

Summary

In the first year of Dr. Valberg’s tenure as the McPhail Dressage Chair Dr. Valberg established the Equine Neuromuscular Diagnostic Laboratory at Michigan State University. This laboratory received diagnostic muscle biopsy submissions for 250 horses from across North America in 2016 and, combined with a repository of >3500 previous submissions, served as a tremendous source of research materials. Within our first year, several key advances were made. We discovered a new muscle disease in Warmblood horses, Myofibrillar Myopathy, we discovered the genetic basis for immune mediated myositis which causes reversible muscle wasting, and we developed new diagnostic techniques. The deeper understanding we now have of the symptoms and pathways involved in specific neuromuscular diseases will improve management and treatment of these disorders. We have communicated our advances to veterinarians and horse owners through scientific articles, lay publications and our website which received 40,338 page views in 2016.

https://cvm.msu.edu/research/faculty-research/valberg-laboratory

This report summarizes the research studies we performed at MSU that resulted in submission of a research publication in 2016. Many other research projects are underway and will be reported in 2017 when the data is analyzed and ready for publication.
I. Type 2 Polysaccharide Storage Myopathy

**Background:** Exercise-induced muscle pain causes performance limitations in Warmblood horses with polysaccharide storage myopathy (PSSM) being the most frequently diagnosed muscle disease in North American and European Warmblood horses. We have been able to subdivide PSSM into two types. Type 1 PSSM (PSSM1) is caused by an inherited mutation in the glycogen synthase 1 gene (GYS1). The cause of type 2 PSSM (PSSM2) is unknown and a subject of current research. PSSM2 is diagnosed by excluding the presence of the GYS1 mutation and finding abnormal staining for muscle glycogen in a muscle biopsy.

**Objectives:** The objective of the study was to compare and characterize clinical signs of PSSM1 and PSSM2 in Warmbloods and non-Warmblood horses.

**Methods:** We performed a retrospective analysis of 3,615 clinical muscle biopsy submissions to our diagnostic laboratory of which 581 were Warmblood horses primarily used for dressage. Symptoms, indicators of muscle damage within the bloodstream (serum CK and AST) and biochemical assessment of muscle glycogen concentrations were compared between PSSM1 and PSSM2 and different breeds.

**Results:** Muscle pain, stiffness and muscle cellular damage (rhabdomyolysis) were the most common symptoms of PSSM1 in both Warmbloods and non-Warmbloods and of PSSM2 in other non-Warmblood breeds. The most common symptom of PSSM2 in Warmbloods was a mild, poorly localized, hindlimb lameness and stiffness. Muscle glycogen concentrations and blood indicators of muscle damage were significantly higher in Warmblood and non-Warmblood with PSSM1 than Warmblood with PSSM2. Normal Warmbloods and PSSM2 Warmbloods had the same concentrations of muscle glycogen.

**Conclusions and relevance:** While PSSM1 in Warmblood and non-Warmblood horses is characterized by tying up, high muscle glycogen concentrations and high indicators of muscle cell damage, PSSM2 in Warmblood horses is characterized by stiffness and vague lameness, normal muscle glycogen concentrations and little indication of muscle cell damage (CK and AST). It is important for veterinarians to recognize the difference in symptoms of PSSM1 and PSSM2 in Warmblood horses in order to include PSSM2 as a potential rule out for back soreness, vague lameness and stiffness in Warmblood horses.

**Data presentation:** Presented at 2016 American Association of Equine Practitioners, 2016 American College of Veterinary Internal Medicine, MSU research Day

**Scientific Publication:** accepted American Journal of Veterinary Research

**Awards:** Zoe Williams: Outstanding research presentation by a freshman veterinary student at MSU Research Day Oct 2016

**Lay publications:**
Article in press with ‘The Horse” magazine
Information available to the public at: https://cvm.msu.edu/research/faculty-research/valberg-laboratory/type-2-polysaccharide-storage-myopathy

Planned: My horse University online presentation
II. Myofibrillar Myopathy (MFM)

Background: We recently identified a new muscle disease in Arabian horses we called myofibrillar myopathy (MFM) and strongly suspected that this disease was present in Warmblood horses with signs of muscle pain and back soreness.

Objectives. 1) To use new light and electron microscopic techniques to identify and characterize warmblood horses with MFM and to fully describe the symptoms they display. 2) To investigate the possible inheritance of MFM.

Methods: Retrospectively, we identified Warmblood dressage horses with MFM by screening muscle biopsies from our laboratory database with specialized staining techniques. The clinical histories and features of muscle biopsies were summarized for those with signs of MFM. Prospectively, muscle biopsies were obtained from healthy control horses and from a three-generation family of Warmblood horses. Samples were assessed for muscle glycogen concentrations, histopathology, fiber types, cytoskeletal and contractile protein aggregates, and fine structural abnormalities using electron microscopy.

Results: MFM was identified based on desmin staining in Warmblood horses. MFM cases had exercise intolerance, reluctance to go forward, stiffness and poorly localized lameness. Abnormal aggregates of the cytoskeletal protein desmin were found in fast twitch type 2 muscle fibers. The contractile proteins were disrupted focally with Z-disc degeneration, myofibrillar disruption and accumulation of irregular granular material. Muscle glycogen concentrations were similar between MFM cases and controls. In the Warmblood family, desmin positive aggregates indicating MFM were found in muscle fibers of the founding dam and in horses from two subsequent generations.

![Figure 1](image.png)

Figure 1. A desmin stain of skeletal muscle from a horse with MFM is shown on the left. Abnormal red clumping of desmin occurs in scattered muscle fibers of horses with MFM. A normal desmin stain of equine skeletal muscle is shown on the right where a normal amount of desmin is seen under the cell membranes.

Conclusions and relevance: A distinctive and potentially heritable muscle disease called MFM was discovered in Warmblood horses that is characterized by exercise intolerance and a vague abnormal
hindlimb gait. This new disorder may be the basis for muscle soreness and vague lameness in horses that previously lacked a diagnosis for their poor performance.

**Future Directions:** We are working to determine if this is a genetic disorder, what the basis for MFM is in horses and to develop rehabilitation exercises and diets to improve the performance of horses with MFM.

**Data presentation:** Presented at 2016 American Association of Equine Practitioners, 2016 American College of Veterinary Internal Medicine

**Scientific Publication:** submitted to Equine Veterinary Journal

**Lay publication:** designed and ready to be posted on our website when the publications is accepted

III. Type 1 Polysaccharide Storage Myopathy

**Background:** Polysaccharide storage myopathy (PSSM1) is a form of tying up or “exertional rhabdomyolysis (ER)” caused by an autosomal dominant mutation in glycogen synthase 1 (GYS1). A puzzling aspect of this disease is why some horses have severe symptoms, whereas other have no symptoms, yet they possess the same genetic mutation. Disease penetrance is known to be impacted by genotype, diet and exercise. The question to be answered in this study was if a horse inherited the GYS1 genetic mutation on chromosome 10 from the sire rather than the dam would this impact the severity of symptoms expressed.

**Objectives:** 1) to determine how often PSSM1 horses with symptoms of tying up inherited the genetic mutation from the sire vs the dam. 2) to determine if inheriting the genetic mutation from the sire impacted muscle sugar content (glycogen concentration) and/or muscle degeneration.

**Methods:** 1) Registered PSSM1 Quarter Horses were identified from our diagnostic sample submission database and AQHA records were searched to determine whether their sire had PSSM1. 2) A family of horses descending from an asymptomatic sire with PSSM1 were studied using exercise testing, plasma CK and AST activity to indicate muscle damage and muscle biopsy.

**Results:** 1) Horses with tying up inherited the PSSM1 mutation three times more frequently from the dam than the sire. 2) Although no offspring of the asymptomatic stallion exhibited tying up during the exercise test, in offspring that inherited the PSSM1 mutation there was subclinical evidence of muscle degeneration and abnormally high muscle glycogen concentrations.

**Conclusions and relevance:** Clinical signs of tying up appear to occur more often with maternal vs. paternal inheritance of PSSM1. This could possibly be due to silencing of the genetic mutation if inherited from the sire. Inheritance of the PSSM1 mutation from the sire, however, is not benign as we found clear evidence of subclinical muscle damage in asymptomatic PSSM1 offspring of an asymptomatic PSSM1 sire. Additionally 25% of horses with signs of tying up in our database did inherit the GYS1 mutation from their sire so it is less likely but still possible to have signs of tying up if the PSSM1 mutation is inherited from the sire.

**Future directions:** We are currently validating this clinical finding by measuring the actual gene expression in muscle versus blood samples of PSSM1 horses. This is one of the first studies to investigate the impact of parent of origin of an autosomal dominant mutation on clinical disease expression in horses.
**IV. Shivers**

**Background:** Shivers is a gait abnormality affecting Warmblood and draft horses, particularly tall geldings, that causes hyperflexion or hyper extension of the hindlimbs when horses are asked to back up or hold up their limb for the farrier. It gradually causes a decrease in performance and the ability to trim hooves. The basis for this chronic progressive disease is not known and the reason for the hyperflexion of the limbs has remained a mystery.

**Objectives:** To study the activation of the hindlimb muscles of Shivers horses using surface electrodes [electromyographic (EMG)] during various gaits and to correlate gait abnormalities with potential pathological changes in a region of the brain called the cerebellum.

**Methods:** Hindlimb stride abnormalities in draft horses with Shivers and healthy control horses were evaluated during a locomotor exam that including trotting and walking backward and forward. Patterns of activity in sets of flexor and extensor muscles were recorded via surface EMG. EMG signals were filtered, normalized to trotting peak EMG and the ensembles of strides produced were compared between Shivers and normal horses. Post-mortem sections of the cerebellum of Shivers horses were examined with special stains.

**Results:** A loss of temporal precision of muscle firing was apparent in Shivers horses especially during walking backward. Mean peak EMG or iEMG activity were similar in control horses walking backward and walking forward. Mean peak EMG or iEMG activity were much higher during walking backward vs forward in Shivers horses. Abnormal swellings were present in Purkinje axons in the cerebellum of all Shivers horses.

**Conclusion and clinical relevance:** The abnormal backing up and inability to hold up a limb for the farrier in Shivers horses is caused by poorly timed and increased flexor and extensor muscle fiber recruitment as well as a neurodegenerative change in selective Purkinje cells in the cerebellum. This is the first time the basis for abnormal limb movement in Shivers has been identified.
Future directions: We are currently investigating the possible genetic basis for Shivers and potential mechanisms for creating muscle relaxation.

Data presentation: Presented at 2016 Research Day Michigan State University, University of Minnesota, Plant and Animal Genome Meeting Jan 2017

Scientific Publication: submitted Equine Veterinary Journal

Lay publications:


Websites: In pursuit: Solving the mystery of Shivers https://cvm.msu.edu/vetschool-tails/valberg-shivers

https://cvm.msu.edu/research/faculty-research/valberg-laboratory/information-on-shivers

Planned: My horse University online presentation

Awards: Our publication on Shivers histopathology received the 2016 CL Davis Foundation Journal Award Veterinary Pathology for the most innovative and outstanding publication for 2016

2017

In summary, in 2016 research performed at the McPhail Center for Equine Sports Medicine has identified new muscle diseases that undoubtedly impact performance in dressage horses and has more clearly defined their underlying bases. This research forms the foundation for our current studies which are investigating ways to enhance performance of these horses through modulation of diet and exercise regimes and we are working to discover minimally invasive genetic tests to diagnose forms of type 2 PSSM, myofibrillar myopathy and Shivers.