Significant Distal Phalangeal Bone Loss in Equine Laminitis

+Engiles, JB; Galantino-Homer HL; Dishowitz, MI; McDonald D; Boston R; Hankenson, KD

University of Pennsylvania, Philadelphia, PA

engiles@vet.upenn.edu

INTRODUCTION: Laminitis is a prevalent disease condition affecting the hoof of horses that results in significant lameness, morbidity, and mortality. Epidemiology studies performed in the U.S. report laminitis affects from 7.5-15.7% of horses per year; of these 4.7% are euthanized due to laminitis, with an estimated economic cost to the industry ranging from millions to hundreds of millions of dollars. The equine hoof is a specialized keratinized epidermal appendage; analogous to the human nail, that encases the equine distal phalanx (P3). Sensitive epidermal and dermal lamellar tissues attach and suspend the distal phalanx (P3) within the hoof capsule. The blood supply to P3 and the overlying sensitive epidermal/dermal lamellae is shared through a series of perforating vascular canals that extend from the medullary spaces of P3, through the semi-porous dorsal cortex, into the overlying soft tissues.

At the initiation of laminitis there is necrosis and separation of the sensitive lamellae; however chronic disease is characterized by aberrant epidermal lamellar proliferation with metaplasia and hyperkeratosis. Although progression is variable, functional loss of lamellar attachments results in biomechanical failure of the P3-hoof capsule interface with subsequent P3 ventral rotation, distal displacement, and eventual prolapse through the sole. Severe intractable pain and morbidity that ensues often necessitates humane euthanasia.

The pathogenesis of equine laminitis is complex and incompletely understood and to date there is no known cure. Associated risk factors include systemic, local, or orthopedic inflammatory conditions (gastrointestinal disease, pneumonia, retained fetal membranes with endometritis/metritis, vasculitis, endotoxemia, cellulitis), biomechanical “overload” (often due to painful orthopedic disease in the contralateral limb), direct trauma to the foot, excessive carbohydrate ingestion, and endocrine/metabolic disease most often associated with pituitary dysfunction of the pars intermedia (PPID) and/or obesity.

Further surprisingly, bone changes in laminitis are poorly characterized. Preliminary histological evaluation of P3 from laminitis horses indicates changes present in both early (acute/subacute) as well as later stages of disease. We hypothesize that P3 osteopatology is a significant feature of both acute/subacute as well as chronic laminitis. We propose that the unique shared vasculature between the bone and sensitive lamellae facilitates interaction between these tissue interfaces. To better characterize as well as quantify laminitis-associated P3 osteopatology, we designed a study utilizing both microcomputed tomography (microCT) and routine histopathology to examine the microarchitecture of P3 in conjuction with sensitive lamellar tissues from clinically “normal” horses and horses with various stages of laminitis.

METHODS: Parasagittal sections of P3 and sensitive dermal/epidermal lamellar tissues (1.5cm thick) were harvested from 38 feet of 15 necropsied horses (10 with clinical history of laminitis and 5 with no known history of laminitis). Horses (N) were divided into two groups based on use (performance; N=9; 21 feet vs. non-performance; N=6; 15 feet). Laminitis-associated risk factors were recorded for both individual feet (e.g. local inflammatory disease, lameness), as well as the animal (systemic inflammatory disease, obesity or endocrine disease (PPID)). Sections were grossly evaluated for laminitis (1=normal/minimal, 2=mild, 3=moderate, 4=severe), distribution of lesions (1=normal/focal, 2=multifocal, 3=regional, 4=global), and known duration of disease (1=0-5 days, 2=6-14 days, 3=15-30 days, 4=>30 days). Due to size constraints of the vivaCT 40 microCT system (Scanco), P3 was obliquely sub-sectioned into proximal and distal portions, and scanned/analyzed separately. Manually drawn contour lines delineating compact and trabecular bone compartments were used to generate Region of Interest (ROI) for a total of 50 slices at 25µm scanned intervals. Volume morphometric analysis was performed with different threshold parameters (360/245) optimized for detecting equine P3 compact/trabecular bone, respectively. MicroCT measurements were analyzed by Kruskal-Wallis One Way Analysis of Variance on Ranks and pairwise post-hoc tests using STATA11.1 statistical analysis software.

RESULTS: MicroCT morphometrical/volumetrical measurements show statistically significant decreases (p<0.05) within P3 trabecular and compact bone compartments including total bone volume (bv), compact bv, trabecular bv, compact bv/total volume, and other trabecular morphometrics that correlate with severity and progression of gross and histopathologic sensitive lamellar pathology. [Fig. A-D] These changes are observed in both performance and non-performance animals as well as in acute/subacute (radiographically apparent) disease.

DISCUSSION: MicroCT analysis provides new insights about equine laminitis, demonstrating the integration of the keratinized epidermal tissues of the hoof wall with the mineralized mesenchymal tissues of P3. The microCT changes indicate bone loss occurs at an early onset of laminitis disease, and is progressive.

A previous pilot study of decalcified sections of P3 (data not shown) revealed early laminitis-associated histopathological changes in the medullary compartments, which included marrow “edema” and fibroblast spindle cell proliferation, neovascularization, mononuclear inflammation, reactive osteoproliferation, and dramatic osteoclast activation with osteoclastic resorption. The unique microanatomical connections and microcirculation of this specialized musculoskeletal unit provides a direct connection between “bone” and “skin.” Given the recent discoveries pertaining to shared effector molecules and pathways between these two organs, and in diseases such as human psoriatic arthritis and onychodystrophy-associated P3 osteolysis, we speculate that direct relationship exists between these two tissues in laminitic disease.

Further investigation will include histopathological evaluation of decalcified P3 sections and correlation with lamellar transcriptomic/proteomic data acquired from a subset of the subjects.

SIGNIFICANCE: Our study indicates a measurable association of P3 bone loss with equine laminitis. Laminitis-associated activation of bone compartments within P3 likely exacerbates laminitis and/or alters response to clinical therapy.

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