Exploration of Tongue Muscle Pathology in Canine Degenerative Myelopathy

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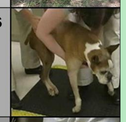
BACKGROUND

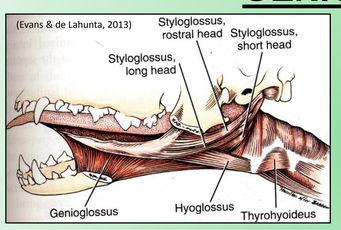
- Canine degenerative myelopathy (DM) is a neurodegenerative disease characterized by late onset and progressive degeneration of motor neurons.
- DM is divided into 4 stages based on severity:

Table 1: Stage of neurological signs in DM dogs

Stage	Neurologic Signs	
1 Early	**UMN Paraparesis** • Progressive general proprioceptive ataxia • Asymmetric spastic paraparesis • Intact spinal reflexes	
2 Early	**Nonambulatory Paraparesis to Paraplegia** • Mild to moderate loss of muscle mass • Reduced to absent spinal reflexes in pelvic limbs • +/- urinary and fecal incontinence	
3 Late	**LMN Paraplegia to Thoracic Limb Paresis** • Signs of thoracic limb paresis • Flaccid paraplegia • Severe loss of muscle mass in pelvic limbs • Urinary and fecal incontinence	
4 Late	**LMN Tetraplegia and Brain Stem Signs** • Flaccid tetraplegia • Difficulty with swallowing and tongue movements • Reduced to absent cutaneous trunci reflex • Generalized and severe loss of muscle mass • Urinary and fecal incontinence	

- DM is a potential disease model to human amyotrophic lateral sclerosis (ALS)
  - Both often occur via a mutation in the *superoxide dismutase 1* gene *(SOD1)*
  - Both cause progressive motor neuron degeneration, resulting in paralysis of muscles involved with limb movement, swallowing, vocalization, and respiration
- DM has previously been shown to cause alterations in muscle fiber composition, size and shape in intercostal muscles, but very little work has been done to determine if histologic changes occur in the orofacial muscles.
- Dysphagia and respiratory distress are two of the major clinical signs reported in ALS. Therefore, it is essential to study how canine DM impacts these vital biological functions.
- The genioglossus is the most affected oral muscle in ALS
  - Its degeneration impedes respiration and swallowing, and increases the likelihood of aspiration.

GENIOGLOSSUS



- Major dilator of the pharynx for inspiration
- Primary muscle for tongue protrusion during inspiration, swallowing & speech
- Composed of 2 major compartments: horizontal & oblique
  - Horizontal: ~2/3 type 1 fibers
  - Oblique: ~1/3 type 1 fibers
- Type 2 myofibers are fast twitch, and are therefore innervated by larger axons, which are more prone to degeneration in ALS
- Type 1 myofibers are slow twitch, and more resistant to degeneration

OBJECTIVE & HYPOTHESIS

The purpose of this study is to investigate the genioglossus of canine DM-affected dogs for evidence of muscle denervation, compared to age- and breed size-matched controls.

We hypothesize the genioglossus will display pathologic changes characteristic of neuromuscular degeneration, with degree of severity correlating to disease stage.

SIGNIFICANCE

- It is difficult to study ALS progression in humans, so a naturally-occurring animal disease model may aid in understanding specific pathogenic mechanisms for ALS. Dogs afflicted with DM are euthanized at various disease stages based on the pet owner's decision. Donation of tissue from these dogs by the owners can help with research on canine DM and on human ALS.
- If the genioglossus of DM-affected dogs is found to have a similar pattern of degeneration as reported for human ALS, this research will provide additional support for canine DM as a disease model for human ALS.

METHODS

Genioglossus Samples:

- Selected 27 samples from our archived collection of immersion-fixed tongues stored in 10% neutral buffered formalin (NBF)
- Categorized based on age, breed size, presence/absence of DM, and stage of DM
- Biopsied up to 8 samples per tongue: 4 from horizontal and 4 from oblique compartments

Genioglossus Biopsies							
Horizontal				Oblique			
Anterior		Posterior		Anterior		Posterior	
Left	Right	Left	Right	Left	Right	Left	Right

Tissue Processing & Slide Preparation:

- Biopsies were stored in fresh 10% NBF for 3-5 days before paraffin processing and embedding
- Each paraffin block contained 4 samples (2 horizontal & 2 oblique) from either the anterior or posterior region of the genioglossus
- Paraffin blocks were cut via microtome into 10 *u*M thick sections and mounted onto silane-coated slides
- Slides were baked in 55^{0}C for 2 hours before staining
- Staining methods are currently in process, including hematoxylin and eosin (H&E) and immunohistochemistry (IHC) targeting various proteins.

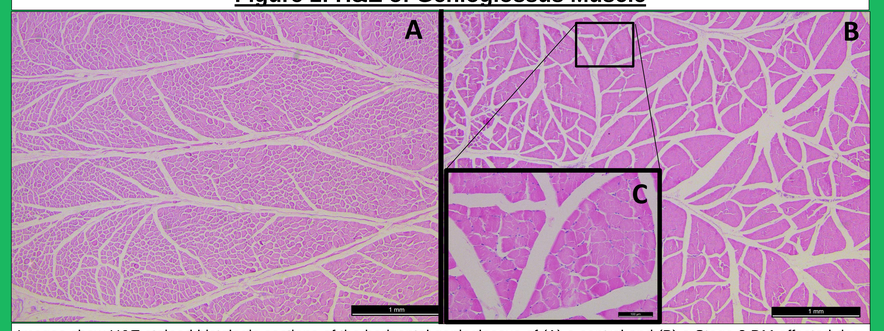
Tissue Examination

- H&E stained slides are currently being examined by light microscopy to identify obvious signs of neuromuscular degeneration

PRELIMINARY RESULTS

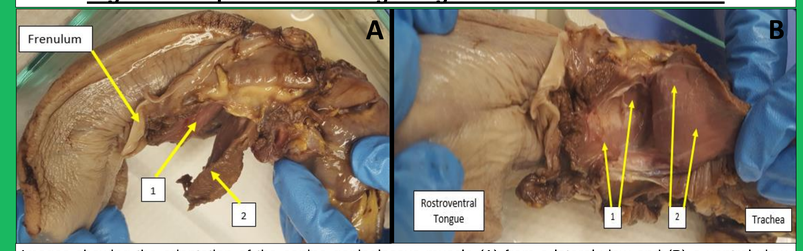
- H&E: general observations:
  - Sections from DM-affected dogs appear to have more condensed myofiber bundles than controls.
  - Vacuoles are present to some degree in all examined slides (DM-affected and control), but appear to be more abundant in samples from older dogs.
  - No apparent nuclear internalization was found.
  - Hyalin fibers were identified in all inspected slides, but were noticed more commonly in older and DM-affected dogs.

Figure 1: H&E of Genioglossus Muscle



Images show H&E stained histologic sections of the horizontal genioglossus of (A) a control and (B) a Stage 3 DM-affected dog. Both are from adults over 9 years of age. The inset (C) shows a 20x view of the boxed region in (B), displaying well-defined peripheral nuclei around distinct myofibers, which are more closely packed than in (A).

Figure 2: Depictions of the genioglossus muscle orientation



Images showing the orientation of the canine genioglossus muscle (A) from a lateral view and (B) a ventral view. This bilateral muscle is composed of an oblique (1) and a horizontal (2) compartment on both the left and right sides.

UPCOMING

- Immunolabeling
  - of type 1 myofibers via IHC to determine the ratio of type 1 vs. type 2 fibers in each region of the genioglossus
  - of SOD1 protein via IHC to analyze the degree, if any, of SOD1 protein aggregation in the muscle tissue of DM-affected dogs
- Measure myofiber diameters
- Quantify potential signs of muscle degeneration (myofiber size changes, nuclear internalization, vacuolization, and presence of hyalin fibers) and differentiate these from similar pathologic changes caused by aging and artifact.
- Analyze data to compare control and DM-affected dogs, and to compare small vs. large breed genioglossus to determine if the muscle's size impacts the results

EXPECTATIONS

This Study:

- We expect late stage DM will cause the genioglossus to have:
  - reduced abundance of type 2 muscle fibers
  - no change or an increase in the number of type 1 fibers
  - aggregation of SOD1 proteins within the myofibers
  - hypotrophic type 2 and hypertrophic type 1 myofibers, as shown by altered fiber diameters

Big Picture:

- If our hypothesis is correct, it would provide a suitable explanation for dysphagia in canine DM and would parallel a mechanism of dysphagia seen in human ALS.

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