Canine DM

- Canine DM is an adult-onset, progressive neurodegenerative disease.
- There are 4 grades based on severity (see Table 1).

<table>
<thead>
<tr>
<th>Table 1: Stage of neurological signs in DM dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
</tr>
<tr>
<td>Early</td>
</tr>
<tr>
<td>Nonambulatory Paraparesis to Paraplegia</td>
</tr>
<tr>
<td>Reduced to absent pinprick and withdrawal in hindlimbs</td>
</tr>
<tr>
<td>Reduced to absent pinprick and withdrawal in forelimbs</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Reduced to absent pinprick and withdrawal in pelvic limbs</td>
</tr>
<tr>
<td>Reduced to absent pinprick and withdrawal in forelimbs</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Reduced to absent pinprick and withdrawal in upper thoracic limbs</td>
</tr>
<tr>
<td>Reduced to absent pinprick and withdrawal in pelvic limbs</td>
</tr>
<tr>
<td>Reduced to absent pinprick and withdrawal in hindlimbs</td>
</tr>
</tbody>
</table>

Methods

- 27 samples from our archived collection of immersion-fixed tongues stored in 10% neutral buffered formalin (NBF).
- Biopsied up to 8 samples per tongue: 4 from horizontal and 4 from oblique compartments.
- 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 and antibodies given to the Veterinary Medical Diagnostic Lab for immunohistochemical staining.

Summary and Limitations

- Increased variability in muscle fiber size and shape occurs as disease progresses.
- Furthermore, the number of type 2 muscle fibers decrease and type 1 fibers hypertrophy.

Significance

- Swallowing and tongue function are eventually affected, regardless of region of onset, in ALS patients. Respiratory failure in the late stages of the disease is the cause of death for most ALS patients, making it difficult to study tissues during disease progression.
- Canine DM paralyses clinical signs of upper motor neuron onset ALS. Furthermore, dogs with DM are euthanized at various stages of disease progression, providing the opportunity to study tissues post-mortem at different disease stages.

Objective and Hypothesis

Our objective is to investigate post-mortem genioglossus samples from age- and breed size-matched controls and DM-affected dogs spanning all disease stages. We hypothesize that the genioglossus muscle will display pathologic changes characteristic of neuromuscular degeneration with degree of severity correlating to disease stage.

Future Directions

- Quantify markers of muscle degeneration (nuclear internalization, vacuolization, reduced abundance of type 2 fibers, and reduced myofiber diameters) in the genioglossus of dogs with DM and age-matched controls.
- Determine which markers of muscle degeneration in the genioglossus readily distinguish DM from aging versus artifact.
- Determine if breed size correlates with myofiber size, such that larger dogs have larger myofibers than smaller dogs. If a correlation exists, then normalization of myofiber size may be warranted for statistical analysis.

References


Acknowledgements

- This project was funded in part by American Boerse Charitable Foundation grant (JRC).
- Thanks to Ria Lambrecht, Lori Lind, and Abbey Lind for their assistance with dissection and staining.
- Acknowledgements to Veterinary Medical Diagnostic Laboratory Staff for assistance with IHC troubleshooting.
- A special thanks to the pet owners who graciously donated their dogs’ tissues to researching DM.
- Student stipend supported by an endowment established by IDEXX-BioResearch.

Preliminary Results

- Figure 4. Immunohistochemical staining of control tissue with anti-slow skeletal myosin heavy chain antibody. This figure depicts representative cross sectional images of the horizontal compartment of the genioglossus. We used Myosin Heavy Chain 1 (MHC-1) antibody to target type 1 myofibers. Fibers positive for MHC-1 appear brown. Samples were counterstained with CAT hematoxylin. Notable tissue features include the nuclei of the myofibers (A), connective tissue (B), individual myofibers (C). (D) is a type 2 fiber and (E) is a type 1 fiber. All samples shown are control tissues stored in formalin for approximately 2 years.

- Figure 5. Immunohistochemical staining of control tissues with myosin heavy chain type 1 antibody (BA-DS). This figure depicts representative cross sectional images of the horizontal compartment of the genioglossus. We used Myosin Heavy Chain 1 (MHC-1) antibody to target type 1 myofibers. Fibers positive for MHC-1 appear brown. Samples were counterstained with CAT hematoxylin. We used BA-DS antibody to target type 1 myofibers. Various pre-treatments were used in an attempt to optimize staining; however, there was no difference in the results. All samples shown are control tissues stored in formalin for approximately 2 years.