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Epidemiological, pathological and metabolomic characterisation of an acquired myopathy of dogs in New Zealand

A thesis presented in partial fulfilment of the requirements for the degree of

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in
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Abstract

‘Go Slow’ myopathy (GSM) is an idiopathic myopathy in dogs in New Zealand, characterised by an acute onset of trembling, weakness and collapse, followed by a prolonged period of exercise intolerance. In the first part of this thesis, the epidemiology of the disease was investigated using a telephone survey to obtain information regarding the diet, exercise, and health of affected dogs. Eighty-six confirmed cases were included in this study, and ingestion of wild pig in the week prior to the onset of clinical signs was a consistent finding (76/86 dogs; 88%; 95% confidence interval = 82 – 95%). Cases occurred most commonly in the upper North Island, particularly in Northland.

The aim of the second part of this thesis was to characterise the pathology of GSM in the same 86 dogs included in the epidemiological study, using serum biochemistry (78 dogs), histology (20 dogs), and electron microscopy (4 dogs). Acutely, affected dogs had increased serum creatine kinase and aspartate aminotransferase activities, corresponding with the histological finding of skeletal muscle degeneration in the absence of inflammation. Ultrastructural changes in skeletal muscle included mitochondrial hypertrophy, intramitochondrial inclusions and increased sarcoplasmic glycogen. Similar lesions were observed in the skeletal muscle of wild pigs from areas where GSM occurred in dogs. Affected dogs also had increased serum alanine aminotransferase activities due to accumulation of lipid and glycogen in hepatocytes. Overall, the microscopic findings were consistent with a toxic myopathy.

To further investigate the pathogenesis of the disease, liver samples were collected from 15 affected dogs and 24 clinically normal dogs for untargeted metabolic profiling using liquid chromatography-mass spectrometry. Comparison of spectra between affected and normal dogs revealed a widespread decrease in phospholipids, and increases in selected dicarboxylic acids and N-acetylated branch chain amino acids in affected dogs. No causative compounds were identified although several candidate mass spectrometric features were identified for future investigation.
Taken together, the results of these studies suggest that ‘Go Slow’ myopathy is a toxic mitochondrial myopathy in dogs that is associated with the ingestion of wild pork. The findings reported aid in the prevention, diagnosis, and management of cases, with the primary suggestion being that owners avoid feeding wild pork in areas where the myopathy occurs. Further work is required to elucidate the cause of this disease.
Acknowledgements

Working towards a PhD is often portrayed to be an arduous and solitary pursuit, but I have been fortunate to have a fantastic team of supervisors and supporters that made the task easier. First and foremost, I wish to thank Wendi Roe, who has provided invaluable advice and support during not only my PhD, but also the pathology residency that preceded it. I never imagined myself doing a PhD or having an academic career, but Wendi inspired me to pursue my interests in pathology, research and teaching and I haven’t regretted it for a moment. Many thanks also go to my co-supervisors Brett Gartrell, Nick Cave and Karl Fraser, whose input and encouragement have helped to shape my thesis into what it is. Brett signed up to supervise a PhD on biomarkers of muscle damage in birds, but stayed on as a voice of reason even when I abandoned all the avian aspects of my research – thank you Brett. Nick consistently pushed me to question my findings and explain them in more detail, and although this was frustrating at times, it greatly improved my approach to research and overall level of understanding. I think I have also (finally) learnt the difference between “which” and “that” thanks to Nick. Karl took on the apparently small task of running some mass spectrometry samples for me and ended up teaching someone with very little chemistry knowledge how to process and interpret mass spectral data, for which I am immensely grateful.

This thesis would not have been possible without the many people who helped me recruit cases and collect samples, particularly Jenni Petersen. Jenni is one of the most passionate and determined vets I have ever met, and it is her care and concern for her clients and their animals that led to recognition of ‘Go Slow’ myopathy as a disease that deserved veterinary interest and research. Over the last 3 years, Jenni has spent countless hours talking to (and more importantly, listening to) pig hunters, recruiting cases and collecting samples, and without her dedication and enthusiasm this research would not have happened. I can never thank you enough Jenni, but I can promise to keep working on the ‘Go Slow’ puzzle until all the pieces are in place. Thanks also to the numerous other vets, pathologists, pig hunters and dog owners who willingly shared their time and knowledge to help with this research.
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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>ChE</td>
<td>Cholesteryl ester</td>
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<tr>
<td>Cer</td>
<td>Ceramide</td>
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<tr>
<td>CK</td>
<td>Creatine kinase</td>
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<tr>
<td>cf-DNA</td>
<td>Cell-free DNA</td>
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<tr>
<td>DG</td>
<td>Diglyceride</td>
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<tr>
<td>EM</td>
<td>Electron microscopy</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>FC</td>
<td>Fold change</td>
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<tr>
<td>FDR</td>
<td>False discovery rate</td>
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<tr>
<td>GRMD</td>
<td>Golden Retriever muscular dystrophy</td>
</tr>
<tr>
<td>GPx</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>HE</td>
<td>Haematoxylin and eosin stain</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>miRNA</td>
<td>Micro-RNA</td>
</tr>
<tr>
<td>MPI</td>
<td>Ministry for Primary Industries</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
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<td>neg</td>
<td>Negative ionisation mode</td>
</tr>
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<td>NMR</td>
<td>Nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>PA</td>
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