THE PATHOLOGY OF DEVIL FACIAL TUMOUR DISEASE IN TASMANIAN DEVILS (*SARCOPHILUS HARRISII*).

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A dissertation submitted to Murdoch University for the degree of Master of Philosophy
2006
DECLARATION OF ORIGINALITY

The work described in this thesis is that of the author alone unless otherwise stated in the text. No part of this work has been submitted for any other qualification at this or any other university.

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Murdoch University
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2006
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PREFACE

Give thanks to the creatures of the world for they bring beauty, joy and peace.

We mourn the loss of certain species and pray for the deliverance of endangered ones. Grant them shelter, food, water and fair weather.

Matthew 10:29

For only a few cents you can buy two sparrows, yet not one sparrow falls to the ground without your Father’s consent.

The study of things caused precedes the study of the cause of things.

Richmond Loh
Murdoch University
Western Australia
2006
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ABSTRACT

The pathology of a disfiguring and debilitating fatal disease affecting a high proportion of the wild population of Tasmanian Devils (*Sarcophilus harrisii*) that was discovered is described. The disease, named devil facial tumour disease (DFTD), has been identified in devils found across 60% of the Tasmanian landscape. The prevalence of this disease was extremely variable, possibly reflecting seasonal trapping success. Between 2001 and 2004, 91 DFTD cases were obtained for pathological description. Grossly, the tumours presented as large, solid, soft tissue masses usually with flattened, centrally ulcerated and exudative surfaces. They were typically multi-centric, appearing first in the oral, face or neck regions. Histologically, the tumours were composed of circumscribed to infiltrative nodular aggregates of round to spindle-shaped cells often within a pseudocapsule and divided into lobules by delicate fibrous septae. They were locally aggressive and metastasised in 65% of cases. There was minimal cytological differentiation amongst the tumour cell population under light and electron microscopy. The diagnostic values of a number of immunohistochemical stains were employed to further characterise up to 50 representative cases. They were negative for cytokeratin, epithelial membrane antigen, von Willebrand factor, desmin, glial fibrillary acid protein, CD16, CD57, CD3 and LSP1. DFTD cells were positive for vimentin, S-100, melan A, neuron specific enolase, chromogranin A and synaptophysin. In conclusion, the morphological and immunohistochemical characteristics together with the primary distribution of the neoplasms indicate that DFTD is an undifferentiated neoplasm of neuroendocrine histogenesis.
ACKNOWLEDGMENTS

This project was funded by the Department of Primary Industries & Water and also by the Commonwealth Research Training Scheme, and has been supported by the Australian Wildlife Health Network.

I am deeply indebted to Margaret Williams, my manager at DPIW, for giving me this opportunity to do the work and study.

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It has been a collaborative effort and many bodies and minds have contributed to my effort.

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I would like to thank Dane Hayes especially for all his untiring work in churning out countless histological slides and preparing the samples for electron microscopy. He is a machine!

Jemma Bergfeld and Robyn Sharpe helped collect samples from the field trips to add statistical weight to the pathology investigation (and doing some sight-seeing at the same time).

I would like to thank Ashkan Mahjoor who assisted with the tabulation of histological data and for helping with the selection of relevant immunostains.
I am greatly indebted to Michael Slaven and Gerard Spoelstra for their help and advice on the immunostaining. It was an information mine-field and they helped me develop IHC protocols for the various antibodies and with trouble-shooting.

I also owe my gratitude to the many private veterinary clinicians at clinics in Launceston, Montrose, Kingston, Smithton, Penguin, Devonport and Longford as well as the wildlife parks that have provided samples and cases for examination.

Testing was performed in the Animal Health Laboratories of DPIW Tasmania; Murdoch University, WA; University of Sydney, NSW; AAHL, Victoria and at Royal Hobart Hospital, Tasmania.

Many thanks also go to Catherine Marshall, Alex Hyatt, Jamie Chapman, Andrew Parker, and Peter Fallon for their assistance with histological and electron microscopic examination and interpretations.

Thanks are due to Judy Rainbird, Kathryn Medlock, David Pemberton at Queen Victoria Museum and the Tasmanian Museum and Art Gallery for allowing me to examine the archived materials.

I would like to thank Phillip Clark for his advice on the cytology section.

My heartfelt thanks to Phil Ladds, Brad Chadwick, Roy Mason, Majid Ghoddusi, Karrie Rose, Bruce Rideout, Ray Lowenthal, Tony Ross, Paul Canfield, Jane Sammons, Bruce Jackson, David Obendorf, Paul Tucker, Susan Hemsley, Mark Krockenberger, Rupert Woods, Vanessa Di Giglio, Tim McManus, Philip Nicholls, Sandy Mclachlan, Jo Meers, Rachael Tarlinton, Jon Hanger, Jeff McKee, John Rasko, Chuck Bailey, Tim Holton, Kelly O’Sullivan and David Middleton for providing data and feedback on the results.
The project has also been strongly supported by my colleagues. I am particularly grateful to Nick Mooney, Clare Hawkins, Billie Lazenby, Menna Jones, Heather Hesterman and Jason Wiersma at the Resource Management Branch of DPIW for the collection of samples.

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My parents, John and Agatha, have helped to do the proof reading and my brothers Des and Ray have provided me with a lot of sound advice and encouragement throughout my work.

I would also like to thank Amy for her patience with all my work.

I thank God for making it possible for me to complete this thesis and allowing me to meet so many wonderful people in the process.
PUBLICATIONS ARISING FROM THIS WORK


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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ARWH</td>
<td>Australian Registry of Wildlife Health</td>
</tr>
<tr>
<td>CD</td>
<td>cluster differentiation</td>
</tr>
<tr>
<td>CgA</td>
<td>chromogranin A</td>
</tr>
<tr>
<td>CK</td>
<td>cytokeratin</td>
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<tr>
<td>DAB</td>
<td>3,3'-diaminobenzidine</td>
</tr>
<tr>
<td>DFTD</td>
<td>Devil Facial Tumour Disease</td>
</tr>
<tr>
<td>DFTDH</td>
<td>DFTD Histology score</td>
</tr>
<tr>
<td>DFTHG</td>
<td>DFTD Gross score</td>
</tr>
<tr>
<td>DIW</td>
<td>deionised water</td>
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<tr>
<td>DPIW</td>
<td>Department of Primary Industries &amp; Water</td>
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<tr>
<td>DPX</td>
<td>di-butyl-polystyrene-xylene</td>
</tr>
<tr>
<td>EDTA</td>
<td>(hydroxymethyl) aminomethane</td>
</tr>
<tr>
<td>EMA</td>
<td>ethylenediaminotetraacetic acid</td>
</tr>
<tr>
<td>ES</td>
<td>epithelial membrane antigen</td>
</tr>
<tr>
<td>ES</td>
<td>Ewing's sarcoma</td>
</tr>
<tr>
<td>FFPE</td>
<td>formalin-fixed paraffin-embedded</td>
</tr>
<tr>
<td>GFAP</td>
<td>glial fibrillary acidic protein</td>
</tr>
<tr>
<td>hpf</td>
<td>high power field (equivalent to 400x magnification)</td>
</tr>
<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
</tr>
<tr>
<td>LSP1</td>
<td>leucocyte specific antigen</td>
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<tr>
<td>Mel A</td>
<td>melan A</td>
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<tr>
<td>MGP</td>
<td>methyl green pyronin</td>
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<tr>
<td>MVA</td>
<td>motor vehicle accident</td>
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<tr>
<td>NBF</td>
<td>10% neutral buffered formalin</td>
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<tr>
<td>NET</td>
<td>neuroendocrine tumour</td>
</tr>
<tr>
<td>NSE</td>
<td>neuron specific enolase</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
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<tr>
<td>RMC</td>
<td>Resource Management Branch, DPIW</td>
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<tr>
<td>SMA</td>
<td>smooth muscle actin</td>
</tr>
<tr>
<td>TAHL</td>
<td>Tasmanian Animal Health Laboratory, DPIW</td>
</tr>
<tr>
<td>TBS</td>
<td>tris buffered saline</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TVT</td>
<td>canine transmissible venereal tumour</td>
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<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
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<th>Fig. 1.1</th>
<th>Healthy Tasmanian devils fighting over a carcass, Road-kill and injured animals make up a large proportion of their diet. Picture courtesy of Christo Baars, Netherlands.</th>
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<td>Fig. 2.1</td>
<td>Polyurethane pipes with side slots for ventilation and a sliding trap door are favoured over the traditional metal traps which can cause traumatic injuries to the Tasmanian devils when they try to escape.</td>
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<td>Fig. 2.5</td>
<td>An early case of DFTD in an animal. A small nodule (arrow) located at the rostral part of the chin at the central midline.</td>
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<td>Fig. 2.6</td>
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<td>Fig. 2.7</td>
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<tr>
<td>Fig. 2.8</td>
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Fig. 2.10  Cytological preparation of a fine needle aspirate of DFTD showing anisocytosis. Diff quick. Bar = 25µm.

Fig. 2.11  DFTD in facial skin. The neoplasm occurs in the dermis and present as well circumscribed masses, compressing the surrounding connective tissue. E = epithelium, SC = stratum compactum, H = hair follicle, N = DFTD neoplasm. H&E, Mag x4. Bar = 300µm.

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Fig. 2.14  Necrosis usually appear to occur centrally at first (left) and then progresses peripherally (right). H&E, Mag x4.

Fig. 2.15  Metastatic locations of DFTD (asterisk): submandibular lymph node (a), lung (b), spleen (c), heart (d), ovary (e), rib serosa (f), kidney (g), mammary (h), adrenal (i) and pituitary gland (j). H&E Mag. x4.

Fig. 2.16  Positive reaction on a section of Tasmanian devil intestinal mucosa (inset) and negative reaction on a section of DFTD neoplasm using the Alcian blue technique for acid mucins. Mag. x40.

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Fig. 2.27 Periosteal osteoblastoma. H&E Mag. x10.

Fig. 2.28 Cutaneous lymphosarcoma. H&E Mag. x4.

Fig. 3.1 Positive reaction on a section of duck liver (inset) but negative on a section of DFTD neoplasm using the Congo red technique for amyloid. Mag x40.

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