Cancer in animals: Novel causes and ways of predicting biological behavior

A Collection of Published Papers Presented in Application for the degree of

Doctor of Science

of

Massey University

John S Munday BVSc PhD Diplomate ACVP

15 October 2019

Acknowledgements

The research described in this thesis would not have been possible without the contributions of many people. These include fellow pathologists who provided me with much of the case material that is described in the following papers. While many pathologists have contributed, I am especially grateful for the support of Adrienne French, Isobel Gibson, Rob Fairley, Bronwyn Smits, Bernie Vaastra, and Hania Klobukoswska.

I acknowledge the collaboration, support, and friendship of my first three PhD students who all contributed to the research in the thesis. I have learned much, both professionally and personally, from Danielle Aberdein, Cameron Knight, and Neroli Thomson and I am extremely proud that all three have achieved successful academic careers.

This research includes collaborations with people from many different institutions, but I especially thank Becky Laurie, Frank Frizelle, and Jacqui Keenan for their help and discussions during my research career. I also acknowledge the support of overseas colleagues including Matti Kiupel, Don Meuton, Jim MacLachlan, Patti Pasavento, Jeanine Peters-Kennedy, Richard Malik, Julia Beatty, and Franco Roperto.

I acknowledge the support of colleagues at Massey for helping me during these studies. It is not possible to list everybody, but Evelyn Lupton deserves special thanks for all the slides and PCR scrolls that she has cut for me over the years. I also thank Alistair Johnstone, Mark Collett, Wendi Roe, Keren Dittmer, Matthew Perrott, and Fernanda Castillo-Alcala for making pathobiology such a great group to work in. As far as mentors, Bob Jolly has always been interested in my career and deserves special mention as I would not have written this thesis without Bob's encouragement and support. Keith Thompson has strongly influenced my career by teaching me how to think critically and how to write during my PhD. Probably more importantly, Keith also gave me sufficient flexibility in my PhD to ensure that I really enjoyed research. Keith is also responsible for bringing me back to Massey and his support as an early career academic was invaluable.

I also acknowledge the role my parents played in teaching me the value of knowledge and giving me an interest in the world. Finally, none of this would have been nearly so much fun without the love of my partner Gillian and children Holly and Harry.

Introduction

This thesis for examination for the Doctor of Science degree consists of a collection of papers published in international peer-reviewed scientific journals. The work described was predominantly performed over the last 15 years at Massey University. While performing the research described in this thesis, I have also been involved in other non-cancer research projects. Some of the papers that describe this research are listed at the end of the reference list, but are not contained within the thesis.

My interest in cancer in non-human species first began while I was working as a diagnostic pathologist at the University of Georgia. While in this position I became intrigued by the patterns of cancers that developed in different species and breeds of animals. My interest in cancer further developed into my main research focus when I returned to Massey University in 2004. During the subsequent 15 years, the majority of my research has been into the development and biological behavior of cancer in animals. This research remains important as it was pioneering in suggesting that infectious agents could be important causes of cancer in non-human species. Today, numerous studies are published every year further investigating the role of infectious agents in neoplastic diseases of animals. In addition, considering the rapid progression of veterinary oncology in the last 15 years, determining better ways to predict neoplasm behavior is currently an intense field of research in veterinary medicine.

The thesis is divided into three interlinked sections: *The development and biological behavior of neoplasms in non-human species*, *Papillomaviruses as a cause of disease in cats*, and *Papillomaviruses as a cause of disease in other animal species*. The research in each of the sections is predominantly described chronologically to illustrate the progression of the science. The reference list and corresponding published papers are arranged following the same order as in the summary.

It is submitted that the publications contained within this thesis represent a significant advance in science and in the understanding of the causes and behavior of cancer in the non-human species. The advances made are maybe best illustrated by my research on papillomaviruses (PVs) as a cause of cancer in cats. When this research started, PVs were

only associated with feline viral plaques which are a rare skin lesion of cats. *Felis catus* papillomavirus (FcaPV) type 1 had been detected in a feline viral plaque and that was the only PV type that was known to infect cats. During the 15 years in which I have been researching PVs in cats, my research has revealed that PVs infect almost all cats. I was the first to detect FcaPV-2 and was able to show that this virus frequently causes cutaneous Bowenoid *in situ* carcinomas (BISCs) as well as most likely causing a proportion of cutaneous basal cell carcinomas and up to a quarter of all cutaneous squamous cell carcinomas (SCCs). To further investigate the role of the PVs in these lesions, an immunohistochemical test to determine which skin cancers are caused by PV infection was developed and validated. By using this immunohistochemical technique it was shown that the PV-induced cutaneous SCCs had a more favorable clinical behavior than non-PV induced SCCs. The expression of FcaPV-2 genes within the SCCs was detected and a vaccine that stimulates a strong immunogenic response against this PV type was developed. As part of my research, four novel PV types that infect cats were detected and FcaPV types 3, 4, and 5 were fully sequenced and classified along with *Bos taurus* papillomavirus (BPV) type 14, the apparent cause of feline sarcoids. In the past 15 years I have become the world expert on PV diseases in companion animals and my work has been widely cited. Additionally, I have been invited to write 5 reviews and 7 book chapters and I have presented my findings as an invited keynote speaker at conferences in North America, Europe, and Australia.

Section 1. The development and biological behavior of neoplasms in non-human species

While working as a diagnostic pathologist at the University of Georgia, I noticed that uterine neoplasms were very common in Vietnamese pot-bellied pigs. People kept this smaller breed of pig as pets and many of the older female pigs developing uterine neoplasms. As these tumors showed many similarities to uterine tumors in humans, it appeared possible that they could develop by similar mechanisms.[1,2] Also around this time, subcutaneous fibrosarcomas were being frequently diagnosed in ferrets. These cases appeared to be linked to the use of rabies vaccines, an association that had only previously been suggested in cats.[3] While at the University of Georgia, multiple pathologists collaborated to show that inter-pathologist variability was a significant problem when using subjective criteria to predict the likely behavior of cancer.[4,5]

After returning to Massey in 2014, I was intrigued by the high rate of small intestinal adenocarcinomas reported in sheep in New Zealand. This high rate was especially interesting considering the high rate of bowel cancer suffered by New Zealanders. The potential for a link between the sheep and human cancers had been recognized over 50 years ago, although well-designed epidemiological studies had not definitively linked any environmental factor to these cancers. My first studies defined these neoplasms using histology and immunohistochemistry, allowing their genetic pathways and behavior to be compared to human bowel cancers.[6,7] Subsequent studies did not reveal either an association between any infectious agents or the presence in sheep of a genetic defect that often predisposes people to bowel cancer and the cause of the high rate of cancer in sheep in New Zealand was not determined.[8,9] More recently, along with researchers at the University of Otago, the role of toxin-producing strains of *Bacteroides fragilis* in the development of intestinal cancer of sheep has been investigated,[10] although this research is currently at an early stage and no definitive conclusions are possible.

In addition to the studies on sheep, I have also completed studies investigating whether the lack of rabies vaccination in New Zealand influences the development of injection site sarcomas in cats[11,12] and studies to try to identify genetic defects that predispose to mast cell tumors in dogs[13] and lymphoma in cats.[14] Additionally, a family of

dogs that were predisposed to developing gastric cancer were investigated and found to have an inherited disease that was similar to Ménétrier's disease in people.[15]

My research interest in the use of markers within a neoplasm to predict cancer behavior started in 2013. Cats with nasal planum squamous cell carcinomas (SCCs) that had high levels of p16 protein were shown to have longer survival times than cats with nasal planum SCC with low p16 protein.[16] Subsequent studies revealed that histological and immunohistochemical features can be used to predict the survival time of cats with oral SCCs [17,18] and dogs with mammary gland adenocarcinomas.[19] In 2018 I was invited by Professor Donald Meuten, one of the world's leading experts in veterinary cancer pathology, to contribute to an editorial discussing the use of histology to predict neoplasm behavior.[20]

References

1 **Munday JS**, Stedman NL. Uterine leiomyomas in two Vietnamese pot-bellied pigs (Sus scrofa). Vet Pathol 2002; 39: 580-3.

2 Harmon BG, **Munday JS**, Crane MM. Diffuse cystic endometrial hyperplasia and metastatic endometrial adenocarcinoma in a vietnamese pot-bellied pig (Sus scrofa). J Vet Diagn Invest 2004; 16: 587-9.

3 **Munday JS**, Stedman NL, Richey LJ. Histology and immunohistochemistry of seven ferret vaccination-site fibrosarcomas. Vet Pathol 2003; 40: 288-93.

4 Northrup NC, Harmon BG, Gieger TL, Brown CA, Carmichael KP, Garcia A, Latimer KS, **Munday JS**, Rakich PM, Richey LJ, Stedman NL, Cheng AL, Howerth EW. Variation among pathologists in histologic grading of canine cutaneous mast cell tumors. J Vet Diagn Invest 2005; 17: 245-8.

5 Northrup NC, Howerth EW, Harmon BG, Brown CA, Carmicheal KP, Garcia AP, Latimer KS, **Munday JS**, Rakich PM, Richey LJ, Stedman NL, Gieger TL. Variation among pathologists in the histologic grading of canine cutaneous mast cell tumors with uniform use of a single grading reference. J Vet Diagn Invest 2005; 17: 561-4.

6 **Munday JS**, Brennan MM, Jaber AM, Kiupel M. Ovine intestinal adenocarcinomas: histologic and phenotypic comparison with human colon cancer. Comp Med 2006; 56: 136-41.

7 **Munday JS**, Brennan MM, Kiupel M. Altered expression of beta-catenin, E-cadherin, cycloxygenase-2, and p53 protein by ovine intestinal adenocarcinoma cells. Vet Pathol 2006; 43: 613-21.

8 **Munday JS**, Frizelle FA, Whitehead MR. Mismatch repair protein expression in ovine intestinal adenocarcinomas. Vet Pathol 2008; 45: 3-6.

9 **Munday JS**, Keenan JI, Beaugie CR, Sugiarto H. Ovine small intestinal adenocarcinomas are not associated with infection by herpesviruses, Helicobacter species or Mycobacterium avium subspecies paratuberculosis. J Comp Pathol 2009; 140: 177-81.

10 Keenan JI, Aitchison A, Pearson JF, Frizelle FA, **Munday JS**. Detection of the Bacteroides fragilis toxin gene in sheep with and without small intestinal adenocarcinoma. N Z Vet J 2019; 1-9.

11 Aberdein D, **Munday JS**, Dyer CB, Knight CG, French AF, Gibson IR. Comparison of the histology and immunohistochemistry of vaccination-site and non-vaccination-site sarcomas from cats in New Zealand. N Z Vet J 2007; 55: 203-7.

12 **Munday JS**, Banyay K, Aberdein D, French AF. Development of an injection site sarcoma shortly after meloxicam injection in an unvaccinated cat. J Feline Med Surg 2011; 13: 988-91.

13 **Munday JS**, French AF, Gibson IR, Gwynne K. Widespread mismatch repair protein expression in canine cutaneous mast cell tumors. Vet Pathol 2009; 46: 227-32.
14 Aberdein D, **Munday JS**, Howe L, French AF, Gibson IR. Widespread mismatch repair expression in feline small intestinal lymphomas. J Comp Pathol 2012; 147: 24-30.
15 **Munday JS**, Aberdein D, Cullen GD, French AF. Menetrier disease and gastric adenocarcinoma in 3 Cairn terrier littermates. Vet Pathol 2012; 49: 1028-31.
16 **Munday JS**, French AF, Gibson IR, Knight CG. The presence of $p16^{(CDKN2A)}$ protein immunostaining within feline nasal planum squamous cell carcinomas is associated with an increased survival time and the presence of papillomaviral DNA. Vet Pathol 2013; 50: 269-73.
17 Klobukowska HJ, **Munday JS**. High numbers of stromal cancer-associated fibroblasts are associated with a shorter survival time in cats with oral squamous cell carcinoma. Vet Pathol 2016; 53: 1124-30.
18 **Munday JS**, He Y, Aberdein D, Klobukowska HJ. Increased $p16^{(CDKN2A)}$, but not p53, immunostaining is predictive of longer survival time in cats with oral squamous cell carcinomas. Vet J 2019; 248: 64-70.
19 **Munday JS**, Ariyarathna H, Aberdein D, Thomson NA. Immunostaining for p53 and $p16^{(CDKN2A)}$ protein is not predictive of prognosis for dogs with malignant mammary gland neoplasms. Vet Sci 2019; 6:
20 Meuten D, **Munday JS**, Hauck M. Time to standardize? Time to validate? Vet Pathol 2018; 55: 195-9.

Selected other research publications (out of a total of 157)

78 **Munday JS**, Thompson LJ. Brodifacoum toxicosis in two neonatal puppies. Vet Pathol 2003; 40: 216-9.

79 Munday R, **Munday JS**. Comparative haemolytic activity of bis(phenylmethyl) disulphide, bis(phenylethyl) disulphide and bis(phenylpropyl) disulphide in rats. Food Chem Toxicol 2003; 41: 1609-15.

80 Mathur S, Brown CA, Dietrich UM, **Munday JS**, Newell MA, Sheldon SE, Cartier LM, Brown SA. Evaluation of a technique of inducing hypertensive renal insufficiency in cats. Am J Vet Res 2004; 65: 1006-13.

81 Brown CA, **Munday JS**, Mathur S, Brown SA. Hypertensive encephalopathy in cats with reduced renal function. Vet Pathol 2005; 42: 642-9.

82 **Munday JS**, Bergen DJ, Roe WD. Generalized calcinosis cutis associated with probable leptospirosis in a dog. Vet Dermatol 2005; 16: 401-6.

83 **Munday JS**, Richey LJ, Brown CA, Rodriguez NA, Kiupel M. Extramedullary plasmacytoma of the salivary gland in two Syrian hamsters (Mesocricetus auratus). Vet Pathol 2005; 42: 819-23.

84 Munday R, Munday CM, **Munday JS**. Hemolytic anemia and induction of phase II detoxification enzymes by diprop-1-enyl sulfide in rats: dose-response study. J Agric Food Chem 2005; 53: 9695-700.

85 Miles CO, Wilkins AL, **Munday JS**, Munday R, Hawkes AD, Jensen DJ, Cooney JM, Beuzenberg V. Production of 7-epi-pectenotoxin-2 seco acid and assessment of its acute toxicity to mice. J Agric Food Chem 2006; 54: 1530-4.

86 **Munday JS**, Boston SE, Owen MC, French AF, Aberdein D. Lameness in a dog caused by thoracic wall invasion by a pulmonary neoplasm. J Vet Med A Physiol Pathol Clin Med 2006; 53: 288-92.

87 **Munday JS**, Dyer CB, Hartman AC, Orbell GM. A possible predisposition to dilated cardiomyopathy in Huntaway dogs. N Z Vet J 2006; 54: 231-4.

88 **Munday JS**, Laven RA, Orbell GM, Pandey SK. Meningoencephalitis in an adult cow due to Mortierella woifli. J Vet Diagn Invest 2006; 18: 619-22.

89 **Munday JS**, French AF, Smith A, Wang J, Squires RA. Probable malignant catarrhal fever presented as transient generalised crusting dermatitis in a cow. N Z Vet J 2008; 56: 89-93.

90 **Munday JS**, Thompson D, Finch SC, Babu JV, Wilkins AL, di Menna ME, Miles CO. Presumptive tremorgenic mycotoxicosis in a dog in New Zealand, after eating mouldy walnuts. N Z Vet J 2008; 56: 145-8.

91 Munday R, Mhawech-Fauceglia P, Munday CM, Paonessa JD, Tang L, **Munday JS**, Lister C, Wilson P, Fahey JW, Davis W, Zhang Y. Inhibition of urinary bladder carcinogenesis by broccoli sprouts. Cancer Res 2008; 68: 1593-600.

92 **Munday JS**, French AF, McKerchar GR. Black-hair follicular dysplasia in a New Zealand Huntaway Dog. N Z Vet J 2009; 57: 170-2.

93 **Munday JS**, McKinnon H, Aberdein D, Collett MG, Parton K, Thompson KG. Cystitis, pyelonephritis, and urolithiasis in rats accidentally fed a diet deficient in vitamin A. J Am Assoc Lab Anim Sci 2009; 48: 790-4.

94 **Munday JS**, Wolfe AG, Lawrence KE, Pandey SK. Disseminated Mortierella wolfii infection in a neonatal calf. N Z Vet J 2010; 58: 62-3.

95 Potter KA, Weston JF, **Munday JS**, Johnstone AC. Spina bifida with associated malformations of the central nervous system in Dorper-cross sheep. N Z Vet J 2010; 58: 315-8.

96 **Munday JS**, French AF, Martin SJ. Cutaneous malignant melanoma in an 11-month-old Russian blue cat. N Z Vet J 2011; 59: 143-6.

97 Bazin E, Huet S, Jarry G, Le Hegarat L, **Munday JS**, Humpage AR, Fessard V. Cytotoxic and genotoxic effects of cylindrospermopsin in mice treated by gavage or intraperitoneal injection. Environ Toxicol 2012; 27: 277-84.