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# **THE ASSOCIATION BETWEEN EQUINE PAPILLOMAVIRUS TYPE 2 AND EQUINE SQUAMOUS CELL CARCINOMAS**

A thesis presented in partial fulfilment  
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Doctor of Philosophy

in

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## ABSTRACT

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Squamous cell carcinomas (SCCs) are malignant epithelial neoplasms affecting most species. Equine SCCs are most common on the penis, where they result in significant welfare and economic costs and frequently necessitate euthanasia. In humans, half of penile SCCs are caused by infection with papillomaviruses (PVs). The research described in this thesis investigated whether PVs similarly cause equine penile SCCs (EPSCCs).

Testing of equine penile samples using conventional PCR and consensus primers amplified PV DNA significantly more frequently from SCCs than from non-SCC lesions. Sequencing of the amplified DNA showed that there was just one PV type present, and that it was a newly-discovered PV called equine papillomavirus type 2 (EcPV-2). *In situ* hybridization and immunohistochemistry localized PV DNA and antigen to neoplastic cells but not to adjacent tissue. These results suggested that EcPV-2 could influence the development of EPSCCs.

A quantitative PCR assay was then developed to test for EcPV-2 presence and load in a large number of equine samples from the penis and from other SCC-prone body sites. This showed that EcPV-2 is present significantly more frequently, and at significantly higher loads, in EPSCCs than in non-SCC tissues. Furthermore, some equine pharyngeal SCCs contained low EcPV-2 loads. However, as EcPV-2 was also sometimes present in grossly normal pharyngeal samples, the significance of this was uncertain. EcPV-2 DNA was only rarely detectable in grossly normal vulvovestibular mucosal samples and never in nictitating membrane samples.

To help determine whether EcPV-2 causes cancer or is an incidental bystander, immunostaining for three cellular regulatory proteins (transformation-related protein 53 (p53), retinoblastoma protein (pRb), and cyclin-dependent kinase inhibitor 2A) was performed. This showed that, unlike high-risk human PVs, the presence of EcPV-2 DNA within a SCC was not associated with degradation of the tumor suppressor proteins

p53 or pRb. While these results do not support a causative association between EcPV-2 and equine SCCs, the possibility that EcPV-2 causes cancer by changing other cell regulatory proteins cannot be excluded.

Overall, evidence from our and others' research strongly suggests that EcPV-2 is involved in EPSCCs, but does not prove unequivocally that it causes these neoplasms.

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Finally, in chapter three I wrote, "*One veterinarian contacted me after seeing Horse A, a 9-year-old Standardbred cross gelding ...*" The veterinarian was my wife, Brielle. Her observation, curiosity and willingness to biopsy on my behalf provided me with New Zealand's first recognized case of EcPV-2 infection, which in many ways allowed this thesis to progress past chapter 3. That alone would have been enough, but it was her endless and loving support that let me reach the end, and I need to thank her more than anyone else. I could never have done this without her. While I worked on this thesis, she finished her own PhD, gave birth to and reared our two fantastic children, moved us to Canada, and looked after me more than I deserve. She never stops smiling, and she brightens life for everyone around her. Thank you, Brielle.

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