

**An investigation of the association between
herpesviruses and respiratory disease in racehorses
in Western Australia**

This thesis is presented for the degree of Doctor of Philosophy at Murdoch University

by

Liping Wang

2003

I declare that this thesis is my own account of my research and contains work which has not previously been submitted for a degree at any tertiary education institution

Liping Wang

Table of contents

Abstract		i
Acknowledgements		iii
Chapter 1	General introduction	1
Chapter 2	Literature review	5
Chapter 3	A nested multiplex PCR for the detection and differentiation of equine herpesvirus 1, 2, 4 and 5.	73
Chapter 4	The prevalence of equine herpesviruses in clinically normal horses	90
Chapter 5	The association of equine herpesviruses with respiratory disease and poor performance in adult horses in Western Australia	105
Chapter 6	Evidence for the association of EHV2 with respiratory disease in young horses	129
Chapter 7	General discussion	154
References		164

Abstract

Respiratory disease is an important cause of wastage in the Australian horse racing industry and viruses are frequently suspected as aetiological agents of respiratory disease or poor performance by clinicians and trainers but confirmation is seldom attempted. This thesis deals with the potential role of equine herpesvirus types 1, 2, 4 and 5 in upper respiratory disease and poor performance in horses in Western Australia.

The methodology selected for the identification of equine herpesviruses in tissues of horses was polymerase chain reaction (PCR) and therefore individual PCR assays were developed for the detection of each herpesvirus, and then a nested multiplex PCR was developed to detect all four viruses. There was good correlation between the multiplex PCR for the detection of EHV and the detection of virus by isolation in cell culture, although a combination of the 2 techniques provided greater sensitivity than either technique alone. The multiplex PCR described appeared equally sensitive as specific PCR assays using a single set of primers for each individual virus but reduced labour and reagent costs.

As latency is a well recognised phenomenon in the equine herpesviruses and the horse is subjected to a number of stresses which might induce reactivation of latent infections, it was hypothesised that there would be a background level of replication of the equine herpesviruses in clinically normal horses. Nasal swabs and peripheral blood leukocytes (PBL) were obtained from 282 clinical normal horses and examined for EHV. The results clearly demonstrated the widespread occurrence of EHV in the clinically healthy horses. The rate of detection of different types of EHV varied, as did the prevalence in young and adult horses. The most common EHV detected was EHV5: in 83.2% of 131 of horses <2 years of age; in 40% of horses >2 years of age.

A prospective clinical study was conducted whereby respiratory tract samples and PBL from adult horses with respiratory disease and/or poor performance were

examined for equine herpesviruses; the aim was to determine a possible association between equine herpesvirus infection and respiratory disease and/or poor performance. The relative incidence of factors identified in the history, signalment, physical and laboratory evaluation of horses in the study population was compared between horses from which EHV was identified in respiratory samples and horses negative for equine herpesvirus. The results indicated that equine herpesviruses were important causes of respiratory disease in the study population, and that haematological and cytological data were a poor indicator of such equine herpesvirus infection.

The occurrence of equine herpesvirus in nasal swabs and PBL of weaned or unweaned foals from Thoroughbred breeding establishments was determined and provided data on the occurrence of EHV in association with respiratory disease. EHV5 was detected in nasal swabs and/or PBL at a high prevalence rate in healthy foals and yearling horses but its occurrence was not associated with clinical signs of respiratory disease. In contrast, EHV2 was detected more commonly in nasal swabs and/or PBL from foals with respiratory disease than in similar samples from healthy horses. Experimental infection of 8 horses with EHV2 was attempted and induced clinical signs of respiratory disease, but less severe than observed in the epidemiological studies. The results suggested that EHV2 is associated with mild upper respiratory tract infection in young horses.

Acknowledgements

There are many people who have helped me in one way or another during my PhD program. I wish to thank them all. However, I feel it necessary to mention some of them particularly, and to acknowledge their contribution to my PhD program and thesis.

I am greatly indebted to my supervisor Professor Graham Wilcox for his all understanding support, patient guidance and encouragement during the course of my studies, and especially his assistance in the preparation of this thesis. More than that, I would like to give my heartfelt thanks to him for agreeing to supervise me during my PhD program, at a time when I thought I might not be able to otherwise proceed with postgraduate study.

I also express my special thanks to my co-supervisor Dr Sharanne Raidal. Her helpful explanations and discussions of equine medicine, her kind help in guiding me in the collation of my data, and her tireless work in helping me obtain horse samples and conducting the horse experiments are sincerely appreciated.

I would like to express many thanks to all the members in the animal virology group past and present for their assistance and friendship: Evan Burkala, Stephen Wareing, Mohammad Bassami, Meredith Stewart, Warren Raye, Ingrid Ypelaar, Carol Sheridan, Inna Narayani, Christine Payne and Andrea Pizzirani.

I also would like to thank Ms Sandy MacPhail and Mr David Lines for their technical assistance and support. Many thanks also to Dr David Berryman and Ms Frances Brigg in the State Agricultural Biotechnology Centre, and to the staff in the Division of Veterinary and Biomedical Sciences, for the help and friendship they provided.

I am extremely grateful to Murdoch University for providing me with a Murdoch University Research Scholarship, and to the Rural and Industries Research and Development Corporation (Horse) who provided the funding for my project.

For invaluable support, I cannot express enough gratitude to both my parents and parents-in-law for their domestic support and assistance with child care during my PhD program.

Last but not least, my hearty thanks go to my nuclear family: my husband Lingwen, my son James and daughter Linda. I have really appreciated their generous love, understanding, pride and belief in me.