Epidemiology and Diagnosis of Equid Herpesviruses 1 and 4 in horses in New Zealand.

A thesis presented
in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Massey University

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1998
Abstract

Equid herpesvirus 1 (EHV-1) and Equid herpesvirus 4 (EHV-4) are ubiquitous viral pathogens of horses in all major horse-rearing countries in the world. These viruses are associated with four clinical syndromes, respiratory disease, abortion, perinatal disease and neurological disease. Traditional serological tests, such as virus neutralisation and complement fixation, are unable to discriminate between antibodies to EHV-1 and EHV-4. A blocking ELISA test has been developed which showed the potential to be used to screen horses for the presence of specific antibodies to EHV-1.

The blocking ELISA test was shown to be specific, sensitive and repeatable for detecting antibodies to EHV-1 even in the presence of antibodies to EHV-4 when tested with polyclonal monospecific antisera to the two viruses raised in equine foetuses and in sheep. Vaccinal antibodies produced with the subunit vaccine Pneumequine® cannot however be reliably distinguished, by the blocking ELISA, from antibodies produced in natural infections or following the use of whole virus vaccines. Possibly, future genetically engineered vaccines which incorporate only the surface glycoproteins will stimulate the production of antibodies which will not be detected and allow the use of the test to differentiate horses naturally infected from those vaccinated with engineered vaccines.

In a structured serological survey of Thoroughbred horses in New Zealand it was found that about 70% of adult (>24 months old) horses have specific EHV-1 antibodies and are therefore assumed to be latently infected with the virus. The prevalence increased with age, with 29% of 6-12 month old and 48% of 13-24 month old horses having specific EHV-1 antibodies. Gender was found to have a minor effect on the prevalence of specific antibodies, with female horses having slightly higher prevalence than males. In the survey, samples from two different years were tested. There was a slightly higher prevalence in 1995 than 1993, which is believed to be due to an increase in infection particularly in young horses in that
Abstract

year. In a group of young horses sampled monthly from birth, no clinical signs of respiratory
disease were seen but four of the nine foals showed seroconversion to EHV-1 around the
time of weaning.

In an investigation of an outbreak of EHV-1 abortion on a Thoroughbred stud, high levels
of specific EHV-1 antibody were found in sera from four of the six mares that aborted. The
blocking ELISA test would have had considerable diagnostic value following the first
abortions however, high levels of specific antibody were still present in some of the mares a
year later. It was not possible to determine whether this was due to the persistence of these
antibodies or whether it was due to reinfection or reactivation of latent virus.

With tissue from the aborted foetuses it was possible to evaluate the ability of the EHV-1
specific monoclonal antibody, which forms the basis of the blocking ELISA test, to detect
viral antigen in formalin-fixed tissue. After finding a suitable pretreatment involving
microwave irradiation and trypsinisation it was possible to 'unmask' and visualise viral antigen
in formalin-fixed tissue using the monoclonal antibody and an immunoperoxidase detection
system. This provides a useful tool for the direct diagnosis of infection due to EHV-1 in
tissue without the need for virus isolation and subsequent typing.

The specific blocking ELISA and its associated monoclonal antibody, has proven useful both
in the diagnosis of infection due to EHV-1 and in epidemiological studies of the virus. Use
of the test in other countries, particularly where the incidence of abortion and neurological
disease are higher than in New Zealand, would yield valuable information on the prevalence
of the virus in different situations. In addition, the test has application as a management tool
on a horse stud for the segregation of horses latently infected with EHV-1 and those naïve
to the virus as a control measure for the serious sequelae of abortion and neurological
disease.
Acknowledgements

Firstly, I would like to acknowledge the New Zealand Equine Research Foundation for providing the funding which made it possible to carry out this work and also to Massey University for both the financial support and providing the necessary facilities.

I am grateful for the assistance I have received from many people, too many to name individually, both at Massey University and other institutions. My thanks to them all.

I would like to acknowledge the extensive part Dr. Nigel Perkins played in the foetal inoculations and the induction of the mares. Without his expertise this part of my work would not have been possible.

Special thanks also to Marty Johnston, Robin Whitson, Marge Chandler, Sheila Ramsay and Noel Rutherford for their help in looking after the mares and foals; Drs. Richard Turner and Dave Keenan for providing samples from the abortion cases; Dr. Ian Anderson and the staff of the Equine Blood Typing and Research Unit for the use of their records and samples; Dr. Kern Keene for the samples from the unvaccinated horses; Dr. Alan Alexander and the staff of the Animal Health Services for the provision of the sheep and their help with the inoculations and blood collection; Pacific Vet and Merial New Zealand Ltd. for providing the vaccines; Dr. Warren Hunt and the staff of the AgResearch Thoroughbred Research Stud, Flock House for the use of the horses and their assistance with the collection of samples; Dr. Jim Hutton and the Lincoln Animal Health laboratory for the samples from the abortion cases; Dr. Dirk Pfeiffer for the statistical analysis; Mrs Pat Davey and Mrs Pam Slack for cutting and staining the histological sections; my great workmates, Mrs Roz Power, Miss Sheryl Bayliss and Dr. Phil Clark; Mr Malcolm Rice for getting me started in the lab again and the 'Virology Group' for all the discussions.
To my supervisors, Dr. Keith Thompson, Dr. John Lumsden and Dr. Elizabeth Carpenter a special thanks for their support and advice, not only about this work but future directions.

A very special thanks to my parents for their support and constant encouragement.

And last, but by no means least, I would like to thank my chief supervisor Professor Colin Wilks, for his assistance and support, for always making time available and also for his friendship.
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<tbody>
<tr>
<td>ATV</td>
<td>Antibiotic/Trypsin/Versene</td>
</tr>
<tr>
<td>bp</td>
<td>Base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CF</td>
<td>Complement fixing (antibodies)</td>
</tr>
<tr>
<td>CL</td>
<td>Confidence limits</td>
</tr>
<tr>
<td>Con A</td>
<td>Concanavalin A</td>
</tr>
<tr>
<td>CPE</td>
<td>Cytopathic effect</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DAB</td>
<td>3',3' Diaminobenzidine tetrachloride</td>
</tr>
<tr>
<td>EBTRU</td>
<td>Equine Blood Typing and Research Unit</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
</tr>
<tr>
<td>EFK</td>
<td>Equine foetal kidney (cells)</td>
</tr>
<tr>
<td>EHV-1</td>
<td>Equid herpesvirus 1</td>
</tr>
<tr>
<td>EHV-2</td>
<td>Equid herpesvirus 2</td>
</tr>
<tr>
<td>EHV-3</td>
<td>Equid herpesvirus 3</td>
</tr>
<tr>
<td>EHV-4</td>
<td>Equid herpesvirus 4</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbant assay</td>
</tr>
<tr>
<td>FBS</td>
<td>Foetal bovine serum</td>
</tr>
<tr>
<td>gp</td>
<td>Glycoprotein</td>
</tr>
<tr>
<td>HSV-1</td>
<td>Herpes simplex virus 1</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL-2</td>
<td>Interleukin-2</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>kbp</td>
<td>Kilobase pairs</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>LAT</td>
<td>Latency associated transcripts</td>
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<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>2-ME</td>
<td>2-mercaptoethanol</td>
</tr>
<tr>
<td>MEM</td>
<td>Minimal essential media</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>OPD</td>
<td>ortho-phenylenediamine dihydrochloride</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PBL</td>
<td>Peripheral blood leucocyte</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohaemagglutinin</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PSK</td>
<td>Penicillin/Streptomycin/Kanamycin</td>
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<tr>
<td>RK13</td>
<td>Rabbit kidney (cell line)</td>
</tr>
<tr>
<td>SAHRP</td>
<td>Streptavidin horseradish peroxidase</td>
</tr>
<tr>
<td>SCID</td>
<td>Severe combined immunodeficiency (disease)</td>
</tr>
<tr>
<td>SPF</td>
<td>Specific pathogen free</td>
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<tr>
<td>TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Tissue culture infective doses 50%</td>
</tr>
<tr>
<td>TK</td>
<td>Thymidine kinase</td>
</tr>
<tr>
<td>VN</td>
<td>Virus neutralisation</td>
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