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

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in partial fulfilment of the requirements for  
the degree of Doctor of Philosophy at  
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## Abstract

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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

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 fetuses 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.

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# Table of Contents

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Abstract . . . . .	ii
Acknowledgements . . . . .	iv
Table of Contents . . . . .	vi
List of Figures . . . . .	xi
List of Tables . . . . .	xvi
Abbreviations . . . . .	xviii
<b>Chapter 1 Equid Herpesviruses 1 and 4 - a review . . . . .</b>	<b>1</b>
Introduction . . . . .	1
The Viruses . . . . .	3
Clinical Syndromes and Pathogenesis . . . . .	6
Respiratory disease . . . . .	6
Abortion . . . . .	8
Perinatal disease . . . . .	11
Neurological disease . . . . .	12
Immune Response . . . . .	13
Epidemiology . . . . .	19
Vaccination . . . . .	22
Laboratory Diagnosis . . . . .	28
The Blocking ELISA Test . . . . .	32
The Scope of this Thesis . . . . .	34
<b>Chapter 2 Materials and Methods . . . . .</b>	<b>36</b>
Introduction . . . . .	36
Cell Culture . . . . .	36
Virus Propagation . . . . .	37

---

Virus Neutralisation tests . . . . .	37
The Blocking ELISA test . . . . .	38
Monoclonal antibody . . . . .	38
Biotinylation of the monoclonal antibody . . . . .	38
Preparation of antigen . . . . .	39
Optimisation of antigen and biotinylated monoclonal antibody . . . . .	40
Blocking ELISA method . . . . .	40
The Indirect ELISA test . . . . .	42
<b>Chapter 3 Standardisation of the EHV-1 blocking ELISA . . . . .</b>	<b>43</b>
Introduction . . . . .	43
Immunocompetence of the equine foetus . . . . .	44
Materials and Methods . . . . .	48
Antigen preparation for animal immunisation . . . . .	48
Inoculation of equine foetuses . . . . .	51
<i>Animals used</i> . . . . .	51
<i>Inoculation procedure</i> . . . . .	52
<i>Induction of parturition</i> . . . . .	54
<i>Blood collection</i> . . . . .	54
Immunisation of sheep . . . . .	54
Measurement of antibodies . . . . .	55
Lymphocyte proliferation assays . . . . .	55
<i>Preparation of lymphocytes</i> . . . . .	55
<i>Viral antigen and mitogens used</i> . . . . .	56
<i>Assay procedure</i> . . . . .	56
Interleukin-2 assay . . . . .	57
Results . . . . .	59
Equine foetal inoculations . . . . .	59
<i>Antibody levels</i> . . . . .	59
<i>Lymphocyte proliferation</i> . . . . .	65
<i>Interleukin-2 assay</i> . . . . .	68



---

Antibody responses in the sheep .....	72
Discussion .....	77
Equine foetal inoculation .....	77
<i>The safety of the procedure</i> .....	77
<i>Antibody responses</i> .....	78
<i>Cell-mediated immune responses</i> .....	81
<i>Interleukin-2 assay</i> .....	82
Standardisation of the blocking ELISA .....	83
Summary .....	85
<b>Chapter 4 An outbreak of EHV-1 abortion: a case report .....</b>	<b>87</b>
Introduction .....	87
Abortion due to EHV-1 in New Zealand and Australia .....	87
Materials and Methods .....	88
Histopathology .....	88
Virus isolation .....	89
Measurement of antibodies .....	89
Virus typing by immunocytochemistry .....	89
Immunocytochemistry on formalin-fixed cell cultures .....	90
Immunocytochemistry on formalin-fixed tissues .....	91
Field observations and laboratory findings .....	92
History .....	92
Gross pathology .....	93
Histopathology .....	93
Virus isolation .....	95
Typing of isolates by immunocytochemistry in cell culture .....	95
Immunocytochemistry on formalin-fixed cell culture .....	97
Immunocytochemistry on formalin-fixed tissue sections .....	97
Serology .....	99
Discussion .....	106
Identification of the causative agent .....	106
Use of the blocking ELISA as a possible aid to diagnosis .....	106

---

Immunocytochemistry on formalin-fixed tissues . . . . .	108
DNA fingerprinting of the isolates . . . . .	109
The source of the virus . . . . .	110
Protective immunity . . . . .	111
Summary . . . . .	112

## **Chapter 5 Prevalence of antibodies to EHV-1 and EHV-4 in horses in**

<b>New Zealand . . . . .</b>	<b>113</b>
Introduction . . . . .	113
The Kaimanawa wild horses . . . . .	113
Materials and Methods . . . . .	114
Populations sampled . . . . .	114
<i>Unvaccinated adult horses</i> . . . . .	114
<i>1993 and 1995 survey samples</i> . . . . .	115
<i>Monthly samples from a group of foals and their dams</i> . . . . .	116
<i>Samples from the Kaimanawa horses</i> . . . . .	117
Measurement of antibodies . . . . .	117
Statistical analyses . . . . .	117
Results . . . . .	118
Unvaccinated adult horses . . . . .	118
1993 and 1995 survey samples . . . . .	119
The Kaimanawa horses . . . . .	126
Monthly samples from the foals and their dams . . . . .	127
Discussion . . . . .	132
The prevalence of virus neutralising antibodies to EHV-1 and EHV-4 . . . . .	132
The prevalence of specific EHV-1 antibodies . . . . .	132
The age at which horses are infected with EHV-1 . . . . .	136
Summary . . . . .	137

---

<b>Chapter 6 Evaluation of the EHV-1 blocking ELISA</b> .....	<b>139</b>
Introduction .....	139
Materials and Methods .....	140
Vaccination of sheep with commercial vaccines .....	140
Results .....	141
Sensitivity and specificity .....	141
<i>Animals known to have been exposed to EHV-1 or EHV-4</i> ...	141
<i>Correlation with the virus neutralisation test</i> .....	141
<i>Repeatability</i> .....	143
Vaccination of sheep with commercial vaccines .....	144
Discussion .....	148
Sensitivity and specificity .....	148
Repeatability .....	150
Ability of the blocking ELISA to detect vaccinal antibodies .....	151
Summary .....	152
<b>Chapter 7 Summary and General Discussion</b> .....	<b>153</b>
<b>Appendix I Buffers and Solutions</b> .....	<b>161</b>
<b>Appendix II Blocking ELISA controls</b> .....	<b>163</b>
Serum and Plasma comparison .....	163
Repeatability .....	164
<b>Appendix III Virus neutralisation and blocking ELISA results</b> .....	<b>168</b>
Unvaccinated adult horses .....	168
1993 survey .....	170
1995 survey .....	179
Kaimanawa horses .....	189
<b>Bibliography</b> .....	<b>191</b>

## List of Figures

---

<b>Figure 3.1:</b>	Purification of virus by an aqueous two-phase polymer procedure modified from Schloer and Breese, (1982) . . . . .	50
<b>Figure 3.2:</b>	Ultrasound-guided inoculation of the equine foetus, showing the entry of the needle (arrow) into muscle in the shoulder region of the foetus . . . . .	53
<b>Figure 3.3:</b>	Ultrasound-guided inoculation of the equine foetus, showing injection of the viral antigen suspension in adjuvant . . . . .	53
<b>Figure 3.4:</b>	Virus neutralisation titre to EHV-1 (Durham) obtained on the weekly samples from the mares . . . . .	60
<b>Figure 3.5:</b>	Indirect ELISA results for the pre-suckle foal sera . . . . .	61
<b>Figure 3.6:</b>	Blocking ELISA results for the pre-suckle foal sera . . . . .	62
<b>Figure 3.7:</b>	Percent blocking as determined in the blocking ELISA for the three foal sera with good antibody levels . . . . .	63
<b>Figure 3.8:</b>	Percent blocking as determined in the blocking ELISA when the EHV-1 serum (Foal 5) was diluted in antibody-negative serum (Control Foal 1), a low titre EHV-4 serum (Foal 4) and a high titre EHV-4 serum (Foal 6) . . . . .	64
<b>Figure 3.9:</b>	Titration of human recombinant IL-2 with equine lymphocytes . . . . .	68

---

<b>Figure 3.10:</b> Counts obtained in the IL-2 assays for the supernatants from the bulk lymphocyte cultures from the foals . . . . .	69
<b>Figure 3.11:</b> Counts obtained in the IL-2 assays for the supernatants from the bulk lymphocyte cultures from the mares . . . . .	69
<b>Figure 3.12:</b> Sheep inoculated with EHV-1 on two occasions . . . . .	73
<b>Figure 3.13:</b> Sheep inoculated with EHV-4 on two occasions . . . . .	74
<b>Figure 3.14:</b> Sheep inoculated with EHV-1 as the primary and EHV-4 as the secondary inoculum . . . . .	75
<b>Figure 3.15:</b> Sheep inoculated with EHV-4 as the primary and EHV-1 as the secondary inoculum . . . . .	76
<b>Figure 4.1:</b> Age of pregnant mares on the property during the 1994 outbreak with the outcome of the pregnancy in that year shown as live foals (x) and aborted foals (o) . . . . .	92
<b>Figure 4.2:</b> Histopathology of the lung lesions from the 1995 foetus (x210) with necrosis of bronchial epithelial cells and acidophilic intranuclear inclusion bodies (inset x840). (Haematoxylin and Eosin). . . . .	94
<b>Figure 4.3:</b> Histopathology of the liver lesions from the 1995 foetus showing a focus of necrosis and acidophilic inclusion bodies (x840) (Haematoxylin and Eosin) . . . . .	94

- 
- Figure 4.4:** Immunoperoxidase staining of EFK cells infected with EHV-1 (Durham), EHV-4 (Horner) or the lung isolate from the 1994 aborted foetus. Counter stained with blued Mayer's Haematoxylin. (x420). . . . . 96
- Figure 4.5:** Immunoperoxidase staining of formalin-fixed cell cultures before (a) and after pre-treatment (b). Counter stained with blued Mayer's Haematoxylin (x420) . . . . . 97
- Figure 4.6:** Immunoperoxidase staining of formalin-fixed lung from the 1995 aborted foetus. No pre-treatment. Counter stained with blued Mayer's Haematoxylin. (x420) . . . . . 98
- Figure 4.7:** Immunoperoxidase staining of formalin-fixed lung from the 1995 aborted foetus after pre-treatment (x420). Inset shows positive staining of inclusion bodies (x840). Counter stained with blued Mayer's Haematoxylin. . . . . 98
- Figure 4.8:** Blocking ELISA results on diluted serum samples taken on 21/9/94 . . . . . 102
- Figure 4.9:** Blocking ELISA results on diluted serum samples taken on 2/11/94 . . . . . 103
- Figure 4.10:** Blocking ELISA results on diluted serum samples taken on 1/6/95 . . . . . 104
- Figure 4.11:** Blocking ELISA results on diluted serum samples taken on 6/9/95 . . . . . 105

---

<b>Figure 5.1:</b>	Virus neutralisation titre to EHV-1 (Durham) versus number of horses for the group of unvaccinated adult horses . . . . .	118
<b>Figure 5.2:</b>	Per cent blocking, as determined with the blocking ELISA, versus number of horses for the group of unvaccinated adult horses . . . . .	118
<b>Figure 5.3:</b>	Virus neutralisation titre to EHV-1 (Durham) versus number of horses for the 1993 samples . . . . .	124
<b>Figure 5.4:</b>	Per cent blocking, as determined with the blocking ELISA, versus number of horses for the 1993 samples . . . . .	124
<b>Figure 5.5:</b>	Virus neutralisation titre to EHV-1 (Durham) versus number of horses for the 1995 samples . . . . .	125
<b>Figure 5.6:</b>	Per cent blocking, as determined with the blocking ELISA, versus number of horses for the 1995 samples . . . . .	125
<b>Figure 5.7:</b>	Virus neutralisation titre versus number of horses for the Kaimanawa horses . . . . .	126
<b>Figure 5.8:</b>	Per cent blocking, as determined with the blocking ELISA, versus number of horses for the Kaimanawa horses . . . . .	126
<b>Figure 5.9:</b>	Virus neutralisation titre to EHV-1 (Durham) for the monthly samples from the foals . . . . .	128

---

<b>Figure 5.10:</b> Per cent blocking, as determined with the blocking ELISA, for the monthly samples from the foals .....	129
<b>Figure 5.11:</b> Virus neutralisation titre to EHV-1 (Durham) for the monthly samples from the mares .....	130
<b>Figure 5.12:</b> Per cent blocking, as determined with the blocking ELISA, for the monthly samples from the mares .....	131
<b>Figure 6.1:</b> Per cent blocking, as determined by the blocking ELISA, versus number of horses for which the virus neutralisation titre was <2 .....	142
<b>Figure 6.2:</b> Per cent blocking, as determined by the blocking ELISA, versus number of horses for which the virus neutralisation titre was 2 .....	142
<b>Figure 6.3:</b> Per cent blocking, as determined by the blocking ELISA, versus number of horses for which the virus neutralisation titre was $\geq 32$ .....	143
<b>Figure 6.4:</b> Sheep inoculated with Fort Dodge EHV-1/EHV-4 vaccine on two occasions .....	145
<b>Figure 6.5:</b> Sheep inoculated with Pneumabort K +1B <sup>®</sup> vaccine on two occasions .....	146
<b>Figure 6.6:</b> Sheep inoculated with Pneumequine <sup>®</sup> vaccine on two occasions .....	147



## List of Tables

---

<b>Table 3.1:</b>	The development of tissues, cells and the immune response in domestic animals expressed in days gestation . . . . .	45
<b>Table 3.2:</b>	Inoculum received and timing of inoculations for the six foals . . . . .	51
<b>Table 3.3:</b>	Inoculum used for the immunisation of eight sheep to raise polyclonal monospecific antisera . . . . .	55
<b>Table 3.4:</b>	Virus neutralisation titres against EHV-1 (Durham) and EHV-4 (Horner) obtained on the pre-suckle foal sera . . . . .	59
<b>Table 3.5:</b>	Lymphocyte proliferation counts $\pm$ SD (SI) for the foals . . . . .	66
<b>Table 3.6:</b>	Lymphocyte proliferation counts $\pm$ SD (SI) for the mares . . . . .	67
<b>Table 3.7:</b>	Comparison of stimulation indices from the lymphocyte proliferation and IL-2 assays for the mares . . . . .	70
<b>Table 3.8:</b>	Comparison of stimulation indices from the lymphocyte proliferation and IL-2 assays for the foals . . . . .	71
<b>Table 4.1:</b>	Isolation of virus in EFK cell culture from specified tissues of the foetuses aborted in 1994 and 1995 . . . . .	95

---

<b>Table 4.2:</b>	Virus neutralisation titres against EHV-1 (Durham) and EHV-4 (Horner) for samples collected on 21/9/94 and 2/11/94, 16 days and 6 weeks respectively after the last abortion . . . . .	99
<b>Table 4.3:</b>	Virus neutralisation titres against EHV-1 (Durham) for sera collected during 1995 . . . . .	100
<b>Table 5.1:</b>	Number of samples tested from each region of New Zealand when the results for 1993 and 1995 are combined . . . . .	116
<b>Table 5.2:</b>	The effect of age, gender, year and region on the presence of virus neutralisation and specific EHV-1 antibodies for the 1993 and 1995 samples tested . . . . .	120
<b>Table 5.3:</b>	Adjusted odds ratios and 95% confidence limits from the final regression model for the effect of age, gender, year and region on the risk of the presence of virus neutralising antibodies. . . . .	121
<b>Table 5.4:</b>	Adjusted odds ratios and 95% confidence limits from the final regression model for the effect of age, gender, year and region on the risk of the presence of specific EHV-1 antibodies . . . . .	122
<b>Table 6.1:</b>	Summary of animals inoculated or known to have been infected with EHV-1 . . . . .	141

## Abbreviations

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ATV	Antibiotic/Trypsin/Versene
bp	Base pairs
BSA	Bovine serum albumin
CF	Complement fixing (antibodies)
CL	Confidence limits
Con A	Concanavalin A
CPE	Cytopathic effect
CV	Coefficient of variation
DAB	3',3' Diaminobenzidine tetrachloride
EBTRU	Equine Blood Typing and Research Unit
EDTA	Ethylenediamine tetra-acetic acid
EFK	Equine foetal kidney (cells)
EHV-1	Equid herpesvirus 1
EHV-2	Equid herpesvirus 2
EHV-3	Equid herpesvirus 3
EHV-4	Equid herpesvirus 4
ELISA	Enzyme linked immunosorbant assay
FBS	Foetal bovine serum
gp	Glycoprotein
HSV-1	Herpes simplex virus 1
Ig	Immunoglobulin
IL-2	Interleukin-2
IV	Intravenous
kbp	kilobase pairs

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LAT	Latency associated transcripts
mAb	Monoclonal antibody
2-ME	2-mercaptoethanol
MEM	Minimal essential media
MHC	Major Histocompatibility Complex
OD	Optical density
OPD	ortho-phenylenediamine dihydrochloride
OR	Odds ratio
PBL	Peripheral blood leucocyte
PBS	Phosphate buffered saline
PHA	Phytohaemagglutinin
PCR	Polymerase chain reaction
PSK	Penicillin/Streptomycin/Kanamycin
RK13	Rabbit kidney (cell line)
SAHRP	Streptavidin horseradish peroxidase
SCID	Severe combined immunodeficiency (disease)
SPF	Specific pathogen free
TCID <sub>50</sub>	Tissue culture infective doses 50%
TK	Thymidine kinase
VN	Virus neutralisation