



UNIVERSITI PUTRA MALAYSIA

**EQUINE HERPESVIRUS TYPE 4 INFECTION:
SEROEPIDEMIOLOGY, PATHOGENESIS AND THE EFFECT ON
RACING PERFORMANCE**

KAMARUDIN MD ISA

FPV 2002 9

**EQUINE HERPESVIRUS TYPE 4 INFECTION:
SEROEPIDEMIOLOGY, PATHOGENESIS AND THE EFFECT ON
RACING PERFORMANCE**

By

KAMARUDIN MD ISA

**Thesis Submitted to the School of Graduate Studies, Universiti
Putra Malaysia, in Fulfillment of the Requirement for the
Degree of Doctor Philosophy**

July 2002



Abstract of the thesis submitted to the Senate of Universiti Putra
Malaysia in fulfillment of the requirement for the Degree of Doctor of
Philosophy

**EQUINE HERPESVIRUS TYPE 4 INFECTION:
SEROEPIDEMIOLOGY, PATHOGENESIS AND THE EFFECT ON
RACING PERFORMANCE**

By

KAMARUDIN MD ISA

July 2002

Chairman: Prof. Dr. Mohd Zamri Saad

Faculty: Veterinary Medicine

Equine rhinopneumonitis is an equine respiratory disease caused by equine herpesvirus type 4 (EHV-4). This study provides the first information on the disease status in Malaysia. Serological survey conducted on 1,023 blood samples, representing 23% of equid population in Malaysia (including Sabah and Sarawak) reveals a moderate seroprevalence rate of 60%. However, the prevalence ranges between 0 and 100%. The state that has 0% prevalence maintained the ponies as a closed herd in contrast the states that have 100% prevalence, which are active in importing equids.



Sero-prevalence to EHV-4 varies significantly between states, districts, stables, horse and pony types and age but not affected by upgrading of pony blood through cross breeding. Based on the equid types, thoroughbred racehorse has the highest prevalence of 100%, followed by the warm-blooded horse at 46.8% while pony and pony crosses has the lowest prevalence of 36.9%.

Intranasal infection of EHV-4 on serologically negative local yearling ponies results in a disease characterised by clinical signs of nasal discharge and fever. The fever is not typical of the hyperthermia caused by viral infection since the biphasic temperature increment is absent. Transient leukopaenia is absent while the arterial oxygen and carbon dioxide partial pressures are not altered. All the changes reflect the mild nature of the EHV-4 infection.

The histological and ultra-structural examinations of the mucosa of the respiratory tract indicated a substantial damage of the upper respiratory tract and tracheal mucosa. Multifocal erosion and extensive accumulation of serous, mucus and dead cells on epithelial surface have been observed. Changes in the nucleus include swelling, nuclear lysis, nuclear membrane disintegration and dilation of



perinuclei membrane. In the cytoplasm, the changes observed include vacuolar degeneration, mitochondria swelling with disintegrated cristaea and accumulation of fluid in cytocavity.

Following intra-nasal inoculation, the infectious virus is rapidly transported to the upper respiratory tract and primary bronchiole. By day 7 post-infection, expression of antigen in sub-mandibular lymph node is markedly reduced as compared to day 3, suggesting a quick elimination of EHV-4 antigen.

Successful detection of EHV-4 antigen from the nasal swab samples using nested PCR at 24-48 hours post-race provides evidence that racing could reactivate latent infection and increase the risk of pony contracting the disease. The EHV-4 infection is found to have a negative effect on racing performance. Racehorses that are sero-negative had higher chances of improving or maintaining finishing position. The effect is more prominent in pony where sero-positive pony is less likely to win the race.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra
Malaysia sebagai keperluan untuk Ijazah Doktor Falsafah

**JANGKITAN HERPESVIRUS EKUIN JENIS 4:
SEROEPIDEMIOLOGI, PATOGENESIS DAN KESAN KE ATAS
PRESTASI BERLUMBA**

Oleh

KAMARUDIN MD ISA

Julai 2002

Pengerusi: Prof. Dr. Mohd Zamri Saad

Fakulti: Perubatan Veterinar

Rhinopneumonitis ekuin adalah penyakit sistem pernafasan kuda yang disebabkan oleh herpesvirus ekuin jenis 4 (EHV-4). Kajian ini memaparkan maklumat, buat kali pertamanya, mengenai status penyakit ini di Malaysia. Bancian ke atas 1,023 sampel darah yang mewakili 23% populasi ekuid di Malaysia (termasuk Sabah dan Serawak) mendapati seroprevalen yang sederhana pada tahap 60%. Namun begitu, julat seroprevalen adalah luas, daripada 0% hingga



100%. Negeri yang menunjukkan seroprevalen terendah (0%) mempraktikkan pengurusan tertutup sedangkan negeri yang tinggi prevalen (100%) aktif mengimport ekuid.

Seroprevalen adalah berbeza di antara negeri, daerah, kandang, jenis kuda dan usia tetapi tidak dipengaruhi oleh kacukan kuda padi. Berdasarkan jenis kuda, kuda lumba baka thoroughbred mempunyai kadar tertinggi, iaitu 100%, diikuti kuda darah panas lain (46.8%) manakala kuda padi mempunyai kadar terendah (36.9%).

Jangkitan EHV-4 melalui hidung dikalangan kuda padi berumur setahun yang negatif sera menyebabkan penyakit dengan tanda-tanda demam dan berhingus. Akan tetapi demam tidak seperti jangkitan biasa oleh virus di mana peningkatan suhu badan dua kali tidak berlaku. Sel darah putih pula tidak berkurangan dan tekanan separa oksigen dan karbon dioksida darah tidak terjejas. Ini menunjukkan yang jangkitan disebabkan EHV-4 adalah sederhana.

Walau bagaimanapun, pemeriksaan histologi dan mikroskop elektron mendapati lesi pada mukosa salur pernafasan atas agak buruk. Hakisan terjadi di banyak tempat sementara pемendapan

lendiran serta sel mati dipermukaan salur pernafasan hingga ke trakea turut berlaku. Begitu juga perubahan pada nukleus dan sitoplasma sel terjangkit menunjukkan yang sel mengalami degenerasi dan kematian.

Sebaik sahaja virus masuk ke dalam hidung, ia merebak dalam saluran pernafasan atas dan turun sehingga bronki utama. Menjelang hari ketujuh, antigen kelihatan berkurangan di dalam nodus limfa sub-mandibular yang menggambarkan pemusnahan virus yang cepat.

Penyertaan lumba dikenal pasti sebagai faktor yang mengaktifkan jangkitan pendam dan mendedah kuda kepada jangkitan baru. Ini dibuktikan dengan terkesannya antigen EHV-4 dari sampel calitan hidung menggunakan teknik nested PCR. Jangkitan EHV-4 memberi kesan negatif kepada prestasi berlumba. Kuda seronegatif berpeluang tinggi untuk mengekal atau meningkatkan prestasi berbanding kuda seropositif. Kesan jangkitan ke atas kuda padi adalah lebih ketara di mana kuda padi seropositif berkemungkinan rendah untuk menang perlumbaan.



ACKNOWLEDGMENTS

I am grateful to my supervisory committee chairman, Professor Dr. Mohd Zamri Saad for his valuable advice and guidance throughout this study. My sincere thanks are due to Associate Professor Dr. Mohd Azmi Lila and Associate Professor Dr. Noordin Mohamed Mustapha who have provided advice and useful comments.

My appreciation goes to the Public Services Department Malaysia for granting the scholarship and to the Director General Department of Veterinary Services Malaysia for giving me the opportunity to pursue the study and providing ponies for the experiment. I also would like to record my deep appreciation to Dr H.J. Field for his kind assistance and care while I am on a month attachment at the Veterinary School, University of Cambridge.

My sincere thanks are to directors of states veterinary services, turf clubs management and stables owners for their cooperation and assistance in blood sampling for the epidemiological study. I also would like to express my gratitude and appreciation to the followings who have been of great help in my study: Dr Aziz Jamaludin, Dr Md.



Sabri Mohd Yusoff, Dr Yuslan Sanuddin, Dr Bashir Ahmad, Dr Rahim Saibu, Dr Cik Zalina Mohd Zain, En Jamil Abdul Samad, En Mohd Nurul Ikwana Yosminar, En Shukor Ahmad, Cik Azilah Jalil, En Ho Oi Kuan, En Salehuddin, En Ismail Md. Shairi, En Kamarudin Awang Isa and members of the Faculty of Veterinary Medicine for sharing their technical skills and their ever available assistance's.

This study is dedicated to my wife Rohimah Mohd Rashid and my juniors Farid Wakim, Farul Izzat, Fadzlin Syazana and Fareth Azedy for being understanding throughout the study.



I certify that an Examination Committee met on 18th July 2002 to conduct the final examination of Kamarudin Md Isa on his Doctor of Philosophy thesis entitled “ Equine Herpesvirus Type 4 Infection: Seroepidemiology, Pathogenesis and the Effect on Racing Performance” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that candidate be awarded the relevant degree. Members of Examination Committee are as follows:

ABDUL RANI BAHAMAN, Ph.D.

Professor
Faculty of Veterinary Medicine
University Putra Malaysia
(Chairman)

MOHD ZAMRI SAAD, Ph.D.

Professor/Deputy Dean
Faculty of Veterinary Medicine
University Putra Malaysia
(Member)

MOHD AZMI MOHD LILA, Ph.D.

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

NOORDIN MOHAMED MUSTAPHA, Ph.D.

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

HUGH J FIELD, Ph.D.

University of Cambridge,UK
(External Examiner)

SHAMSER MOHAMAD RAMADILI, Ph.D.

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 31 JUL 2002



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy.



AINI IDERIS, Ph.D.

Professor

Dean, School of Graduate Studies

Universiti Putra Malaysia

Date: **12** SEP 2002

DECLARATION

I hereby declare that the dissertation is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



KAMARUDIN MD ISA

Date: 27/07/02

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL SHEETS	x
DECLARATION	xii
LIST OF TABLES	xviii
LIST OF FIGURE	xxi
LIST OF PLATES	xxii
LIST OF ABBREVIATIONS AND SYMBOLS.....	xxv

CHAPTER

1	INTRODUCTION	1
2	LITERATURE REVIEW	8
	2.1. The Virus	8
	2.2. Disease Caused by EHV-4	10
	2.2.1. Respiratory Disease	10
	2.2.2. Non-Respiratory Disease	11
	2.3. Risk Factors Associated With EHV4 Infection ...	13
	2.3.1. Age	13
	2.3.2. Season	14
	2.3.3. Weaning Status of Foals	15
	2.3.4. Exposure	15
	2.3.5. Exercise	16
	2.3.6. Concurrent Infection	16
	2.4. Diagnosis and Identification of EHV4 Infection..	17
	2.4.1. Virus Isolation	17
	2.4.2. Virus Neutralization Test (VNT)	18
	2.4.3. Enzyme-Linked Immunosorbent Assay (ELISA)	18
	2.4.4. Restriction Fragment Patterns (RFPs)	19
	2.4.5. Polymerase Chain Reaction (PCR)	20
	2.4.6. <i>In-Situ</i> PCR	23
	2.4.7. Immunoperoxidase	24
	2.4.8. Agar Gel Immunodiffusion Test (AGID)	25
	2.4.9. Indirect Immunofluorescence Assay (IFFA)	25
	2.4.10. Sensitivity and Specificity of Various Testing Procedures	26
	2.4.10.1. VNT and ELISA	26



	2.4.10.2. The PCR and Isolation	27
	2.4.10.3. Immunoperoxidase and Fluorescent Antibodies	28
	2.4.10.4. ELISA, AGID and CF	28
2.5.	Prevalence of EHV-4 Infection	29
	2.5.1. Sero-prevalence.....	29
	2.5.2. Non-Serological Prevalence	31
2.6.	Pathogenesis of EHV-4 Infection	32
	2.6.1. General	32
	2.6.2. Cell Tropism	34
	2.6.3. The EHV-4 Distribution	35
	2.6.4. Histological Changes	37
	2.6.5. Ultra-Structural Changes	38
	2.6.6. Latency of EHV-4	39
	2.6.7. Reactivation of Latent Infection	41
	2.6.8. Protection Against Re-Infection and Reactivation	43
2.7.	Effect of Respiratory Infection on Gas Tension ..	44
2.8.	Effect of Respiratory Disease on Horse's Performances	46
2.9.	Focus of The Research	46
3	SEROEPIDEMIOLOGY OF EQUINE HERPESVIRUS TYPE 4 (EHV-4) IN MALAYSIA	48
3.1.	Introduction	48
3.2.	Materials and Methods	49
	3.2.1. Sampling Strategy	49
	3.2.2. Samples Collection	52
	3.2.3. Virus Strain and Tissue Culture	53
	3.2.4. Virus Neutralization Test (VNT)	53
	3.2.5. Enzyme-Linked Immunosorbent Assay (ELISA)	54
	3.2.6. Prevalence Estimates	55
	3.2.7. Statistical Analysis	57
3.3.	Results	58
	3.3.1. Overall Sero-prevalence of EHV-4	58
	3.3.2. Sero-prevalence According to States	58
	3.3.3. Sero-prevalence According to Districts	60
	3.3.4. Sero-prevalence According to Stables	62
	3.3.5. Sero-prevalence According to Horse and Pony Types	63
	3.3.6. Sero-prevalence According to Age	63
	3.3.7. Sero-prevalence According to Grade of	



	Pony (effect of cross-breeding)	63
3.4.	Discussion	66
4	EXPERIMENTAL INFECTION OF SEROLOGICALLY NEGATIVE MALAYSIAN PONIES WITH EHV-4: CLINICAL SIGNS, BLOOD LEUKOCYTES, AND BLOOD GAS TENSION	72
4.1.	Introduction	72
4.2.	Materials and Methods	73
	4.2.1. Yearling Ponies	73
	4.2.2. Virus Strain	74
	4.2.3. Experimental Design	75
	4.2.4. Clinical Signs.....	76
	4.2.5. Arterial Blood Collection	76
	4.2.6. Determination of Blood pO ₂ and pCO ₂	77
	4.2.7. Blood Collection for White Blood Cells Parameter	77
	4.2.8. Statistical Analysis	77
4.3.	Results	78
	4.3.1. Clinical Signs	78
	4.3.1.1. Rectal Temperature	78
	4.3.1.2. Nasal Discharge	79
	4.3.2. White Blood Cells Parameters	81
	4.3.3. Arterial Blood Oxygen (pO ₂) and Carbon Dioxide (pCO ₂)	83
4.4.	Discussion	85
5	HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN THE RESPIRATORY MUCOSA OF SEROLOGICALLY NEGATIVE YEARLING PONIES INFECTED WITH EHV-4	92
5.1.	Introduction	92
5.2.	Materials and Methods	94
	5.2.1. Yearling Ponies	94
	5.2.2. Virus Strain	94
	5.2.3. Experimental Design	94
	5.2.4. Sample Collection	95
	5.2.5. Histological Processing	95
	5.2.6. Statistical Analysis	97
	5.2.7. Transmission Electron Microscopy (TEM) Examination	98
	5.2.8. Ultra-Structural Changes	99
5.3.	Results	99



	5.3.1. Histological Changes	99
	5.3.2. Lesion Score	101
	5.3.3. Detection of Virus Using TEM	101
	5.3.4. Ultra-Structural Changes	103
	5.4.4.1. The Nucleus	104
	5.4.4.2. The Cytoplasmic Organelles	104
	5.4. Discussion	105
6	IMMUNOHISTOLOGICAL DETECTION OF VIRAL ANTIGEN EXPRESSION IN YEARLING PONIES INFECTED INTRANASALLY WITH EHV-4	120
	6.1. Introduction	120
	6.2. Materials and Methods	122
	6.2.1. Yearling Ponies	122
	6.2.2. Virus Strain	123
	6.2.3. Experimental Design	123
	6.2.4. Samples Collection	123
	6.2.5. Production of Hyperimmune Serum	123
	6.2.6. Indirect Immunoperoxidase Staining Method	124
	6.2.7. Viral Antigen Expression	125
	6.3. Results	126
	6.3.1. The EHV-4 Virus Expression	126
	6.3.2. Comparison of Antigen Expression Between Day 3 and 7 Post-Infection	127
	6.4. Discussion.....	131
7	DETECTION OF EHV-4 ANTIGEN FROM NASAL SWABS OF WINNERS AND NON-WINNING RACING PONIES USING A NESTED POLYMERASE CHAINREACTION (PCR) TECHNIQUE	136
	7.1. Introduction	136
	7.2. Materials and Methods	137
	7.2.1. Sampling of Ponies	137
	7.2.2. Swab Samples	138
	7.2.3. Blood Collection	138
	7.2.4. Enzyme-Linked Immunosorbent Assay (ELISA)	138
	7.2.5. Isolation of Genomic DNA	139
	7.2.6. Primers	139
	7.2.7. Polymerase Chain Reaction (PCR)	140
	7.2.8. Experimental Design and Statistical	



	Analysis	142
7.3.	Results	143
	7.3.1. PCR and ELISA Results	143
	7.3.2. Relations of PCR and Racing Results	144
	7.3.3. Comparison of PCR and ELISA Results	144
7.4.	Discussion.....	145
8	ANALYSIS ON RACING PERFORMANCE OF HORSES AND PONIES SERO-POSITIVE TO EQUINE HERPES VIRUS TYPE 4	148
8.1.	Introduction	148
8.2.	Material and Methods	150
	8.2.1. Animals	150
	8.2.2. Performance Indicators	151
	8.2.3. Samples Collection	151
	8.2.4. Enzyme-Linked Immunosorbent Assay (ELISA)	152
	8.2.5. Statistical Analysis	152
	8.2.6. Second Observation on Racing Ponies	154
8.3.	Results	154
	8.3.1. Prevalence of EHV-4 infection	154
	8.3.2. Racing Performance of Racehorses	155
	8.4.2.1. Current Finishing Position	155
	8.4.2.2. Comparison Between Current and Previous Finishing Position	155
	8.3.3. Racing Performance of Race Ponies	156
	8.3.4. Repeat Observation of Racing Ponies	160
8.4.	Discussion	161
9	GENERAL DISCUSSION AND CONCLUSION	165
	REFERENCES	174
	VITA	195



LIST OF TABLES

Tables	Page
2.1 Some of the primers used in detecting/differentiating EHV-4 and EHV-1 and their PCR products	22
3.1 Seroprevalence of EHV-4 in Malaysian horse and pony population according to cluster	59
3.2 Seroprevalence of EHV-4 in Malaysian horse and pony population according to states	60
3.3 Sero-prevalence of EHV-4 in Sabah horse and pony populations according to districts	61
3.4 Sero-prevalence of EHV-4 in Kelantan pony population according to districts	62
3.5 Sero-prevalence of EHV-4 in central peninsular Malaysia according to stable	64
3.6 Overall seroprevalence of EHV-4 in Malaysia according to horse and pony types	64
3.7 Effect of age on prevalence of EHV-4 infection detected by virus neutralization test in Sabah equid	65
3.8 Effect of horse blood level of Sabah graded ponies on prevalence of EHV-4 infection	66
4.1 Secretion of nasal discharge from yearling ponies infected with EHV-4	80
4.2 Effect of EHV-4 infection on absolute number of total white blood cells (WBC) of yearling ponies ($\times 10^3/\text{mm}^3$).	82
4.3 Effect of EHV-4 infection on absolute number of total lymphocytes of yearling ponies ($\times 10^3/\text{mm}^3$).....	82
4.4 Effect of EHV-4 infection on arterial blood oxygen	



	partial pressure (pO ₂) of yearling ponies (mmHg)	84
4.5	Effect of EHV-4 infection on arterial blood carbon dioxide partial pressure (pCO ₂) of yearling ponies (mmHg)	84
5.1	Histological lesion scoring of the upper respiratory tract and trachea (Lu et al., 1982)	96
5.2	Lesion indices of upper respiratory tract mucosa of individual yearling pony infected with EHV-4 at days 3 and 7 post-infection	102
5.3	Mean (±SD) lesion indices of upper respiratory tract mucosa of yearling ponies infected with EHV-4 at days 3 and 7 post-infection (DPI)	103
6.1	Expression of EHV-4 viral antigen (Immunoperoxidase staining) in respiratory tract of yearling ponies after intranasal inoculation at 3 rd and 7 th days post-infection	129
7.1	The primers used for amplifying the DNA of EHV-4 From nasal swab samples	140
7.2	The PCR mix for amplifying EHV-4 DNA from nasal swab samples	141
7.3	The relationship of racing results (winner and non-winner) on the PCR results	144
7.4	The relationship between ELISA and PCR results	145
8.1	Mean ELISA OD ₄₅₀ values according to finishing positions in 25 races	157
8.2	Comparison between previous and current racing performance of racehorses in relation to ELISA results against EHV-4	158
8.3	Relation between ELISA optical density and proportion of racehorses that improved or maintained finishing position in the current racing	



	as compared to previous racing	159
8.4	Comparison on the racing performance of race ponies according to the ELISA results against EHV-4	160
8.5	Second observation on independency of winning according the ELISA test results against EHV-4	161



LIST OF FIGURE

Figure	Page
3.1 Sampling clusters for sero-epidemiology study of EHV-4 in Malaysia	51
4.1 Effect of EHV-4 infection on rectal temperature of serologically negative Malaysian yearling ponies. Each bar represents the mean \pm S.D (n=3). Symbols (*) indicate significantly different (P<0.05)	79
6.1 The dissemination and fate of EHV-4 antigen following intranasal inoculation (a proposal).....	135



LIST OF PLATES

Plates	Page
4.1 Photographs of infected yearling pony (Y3) secreting serous nasal discharge and control yearling pony not showing nasal discharge	80
5.1 Photomicrograph of the cranial lobe of lung of a yearling pony 7 days PI by EHV-4 showing normal bronchiole and alveoli (H&E, x40).....	110
5.2 Photomicrograph of the sub-mandibular lymph node of a yearling pony after 3 days PI by EHV-4 showing the medular region with normal looking medullary sinuses and reticular cell network (H&E, x250)	110
5.3 Photomicrograph of the anterior nasal mucosa of a yearling pony after 3 days PI by EHV-4 showing degeneration of the epithelial cells disruptive of mucosal arrangement, and the surface cells that devoid of cilia (H&E, x250).....	111
5.4 Photomicrograph of the middle nasal mucosa of a yearling pony after 3 days PI by EHV-4 showing extensive accumulation of cellular debris, mucus and blood cells on the affected epithelial surface (H&E, x100)	111
5.5 Photomicrograph of the posterior nasal mucosa of a yearling pony showing the stages of epithelium layer damage – almost all epithelium cells on the right side have been eroded exposing the basement membrane while on the left side the ciliated epithelium cells are still intact (H&E, x100)	112
5.6 Photomicrograph of the anterior nasal mucosa of a yearling pony at 7 days PI by EHV-4 showing epithelium layer completely eroded exposing the basement membrane (arrows)	



	(H&E, x100)	112
5.7	Photomicrograph of the middle nasal mucosa of a yearling pony at 7 days PI by EHV-4 showing most of the epithelium layer that has been eroded with dead cells and mucus on the epithelial surface (H&E, x100).....	113
5.8	Photomicrograph of the posterior nasal mucosa of a yearling pony at 7 days PI by EHV-4 showing accumulation of mucus, cell debros and blood cells on the epithelial surface (H&E, x40)	113
5.9	Photomicrograph of the trachea of a yearling pony at 7 days post - infection by EHV-4 showing erosion of the epithelial layer (thin arrow) and accumulation of mucus and cell debris on ciliated epithelial cells (thick arrow) (H&E, x100).....	114
5.10	TEM. Epithelium of nasal mucosa of yearling pony infected with EHV-4, 3 days after infection showing viral particles budding out of nuclear membrane (arrows)	115
5.11	TEM. Epithelium of nasal mucosa of yearling pony infectd wth EHV-4, 3 days after infection showing viral particles in cytoplasmic vacuoles (arrows)..	115
5.12	TEM. Epithelium of nasal mucosa of a yearling pony infected with EHV-4. Nuclei changes, A. Swollen nucleus, with nuclear body (thin arrow), lysis of nucleoplasm and disintegration of nuclei membrane (x8000), B. Dilation of perinuclear space (thick arrow)(x50000).....	116
5.13	TEM. Epithelium of nasal mucosa of yearling pony infected with EHV-4. Nucleus changes, 3 days after infection A. Nuclear swelling in degenerated cell, and B. Nucleus contraction in necrotic cell (x5300)	117
5.14	TEM. Epithelium of the nasal mucosa of a yearling	



	pony infected with EHV-4. Cytoplasmic changes. Accumulation of fluid in the cytocavity including, mitochondria and endoplasmic reticulum (x25000)	
	B. Vacuolar degeneration – accumulation of fluid in vacuoles and swelling of mitochondria (x8000)..	118
5.15	TEM. Epithelium of the nasal mucosa of a yearling Pony infected with EHV-4. Cytoplasmic change, A. Presence of dense granules with swollen mitochondria (x6300), B. Swollen mitochondria-disintegration of cristae (thin arrow) and completely lysed cristae (thick arrow)(x20000)	119
6.1	Photomicrograph of the eroded and detached epithelial cells of posterior nasal mucosa of a yearling pony expressing EHV-4 viral antigen, 7 days PI (IP, x400)	128
6.2	Photomicrograph of the surface of epithelial cells of trachea expressing EHV-4 antigen, 7 days PI (IP, x1000)	128
6.3	Photomicrograph of the medular sinuses of sub-mandibular lymph node showing numerous (arrows) brown colouration expressing the EHV-4 antigen, 3 days PI (IP, x100).....	130
6.4	Photomicrograph of sub-mandibular lymph node of the yearling pony, 3 days PI showing reticular cells (thick arrows) of medulary sinuses and lymphoblast cells (thin arrow) in sub-mandibular expressing EHV-4 antigen (IP, x1000)	130
7.1	Electrophoretic analysis of PCR amplification from nasal swab of racing ponies. Sixteen amplificates (W1, W2, W3, W4, W5, W6, L1, L2, L3, L4, L6, L7, L8, L9, W8 and W9) were positive to EHV-4 with band size of 580 bp and two amplificates (L5 and W7) were negative. Molicular markers (1kb) were denoted as M, and CN was control negative. Ws are winners and Ls are non-winners.....	143

