

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

# **Equine Respiratory Viruses in New Zealand**

*A thesis presented in partial fulfilment of the  
requirements for the degree of  
Doctor of Philosophy  
at Massey University, Turitea, Palmerston North,  
New Zealand*

**Magdalena Dunowska**

**1999**

The outcome of any serious research can only be to  
make two questions grow where only one grew before.  
[Veblen T. *The Place of Science in Modern Civilization* (1919)]

## Abstract

---

Equine respiratory disease is a cause of wastage resulting in financial losses for the equine industry throughout the world. A serological and virological survey was conducted on samples collected from a total of 133 horses from different parts in New Zealand. Three groups of foals were sampled on a monthly basis, five outbreaks of respiratory disease were investigated, and samples were collected from 37 yearlings during and following the yearling sales. The only viruses isolated were equine herpesviruses (EHV) types 2, 5, and 4. EHV-2 was isolated from 99% of peripheral blood leucocyte (PBL) samples from foals sampled on a monthly basis and from PBL of 96% of horses from outbreaks and yearlings from the sales. Additionally, EHV-2, EHV-5 or both were isolated from nasal swabs of up to 100% of foals sampled on a monthly basis between March and July. The time of virus excretion from the nasal cavity varied slightly between the three groups. The rate of virus isolation from the nasal swabs was highest at the time when most foals from two of the groups experienced some respiratory signs. Foals from the remaining group, however, were healthy throughout the study period. Of horses from outbreaks and yearlings from the yearling sales, EHV-2, EHV-5, or both were isolated from nasal swabs of 35% of horses showing respiratory signs, 9.5% of healthy horses, and 37.5% of horses for which individual clinical data were not available. EHV-4 was isolated on only one occasion, from PBL of a foal with respiratory disease.

There was serological evidence that EHV-1, equine adenovirus-1 (EAdV-1), and equine rhinoviruses (ERhV) types 1 and 2 are all present in New Zealand. The average antibody seroprevalence to these viruses was 67%, 61%, 78%, and 13%, respectively. All serum samples tested were negative for antibodies to equine arteritis virus, mammalian reovirus-3 and parainfluenza virus-3. Most of the foals sampled showed serological evidence of infection with EHV-1/4 (78%), EAdV-1 (61%), and ERhV-2 (65%) within their first year of life. There was no indication that any of the foals sampled became infected with ERhV-1 within the period of study. Samples for virus isolation and two blood samples for serology were collected from 54 of 82 (66%) horses sampled from outbreaks and yearlings from the sales for which individual clinical data were available. These included 35 horses showing signs of respiratory disease around the time of sampling and 19 healthy horses. For the remaining 28 horses, either individual clinical data were not available, or the second blood sample for serology was not collected. Recent viral infection was not associated with development of respiratory signs in yearlings from the sales when all viruses were considered, although this result was not statistically significant (adjusted OR 1.3,  $p = 0.5$ ). Equine herpesvirus-2/5 and ERhV-2

infections appeared to be associated with development of clinical signs in yearlings from the yearling sales, although these results were significant only for EHV-2/5, and not ERhV-2. However, since none of the foals or horses sampled was examined endoscopically, it is possible that a number of lower airway infections were not recognised. The most common infection among horses with respiratory signs from outbreaks, for which paired serum samples were available, was EHV-2/5 infection (30.4%), followed by ERhV-2 (13.0%), ERhV-1 (4.3%), and EHV-1/4 (4.3%) infections. None of the 56 horses for which a full set of data were available showed serological evidence of recent EAdV-1 infection and only two horses showed serological evidence of recent ERhV-1 infection. Most horses with signs of respiratory disease that showed serological evidence of recent viral infection also yielded EHV-2 or EHV-5 from their nasal swabs, indicating that EHV-2/5 either predisposes to other infections, or that infection with other viruses re-activates latent EHV-2/5.

During the survey, EHV-5 was isolated on 56 occasions. This represented the first isolation of this virus outside Australia. Representative New Zealand isolates were compared to the reference Australian strain by restriction digest of the cloned *glycoprotein B* gene. Restriction fragment length polymorphism (RFLP) profiles of all but one New Zealand isolate differed from the RFLP pattern of the prototype strain. With few exceptions, isolates from different horses showed different RFLP profiles. However, isolates from individual horses, collected either at different times, from different sites, or grown on different cells showed identical RFLP patterns.

The effect of EHV-2 infection on gene expression in equine leucocytes was investigated by representational difference analysis of cDNA. The results suggested that EHV-2 infection of leucocytes down-regulates the expression of monocyte chemoattractant protein-1. This indicates that EHV-2 has the ability to modulate the chemokine environment of infected cells and may predispose to secondary infections.

This work has contributed to the understanding of factors involved in equine respiratory disease in New Zealand. Although infection with none of the viruses was detected only in horses showing respiratory signs, the results suggest that EHV-2/5 and equine rhinoviruses may be more important than previously thought.

## Acknowledgements

---

I am grateful to the New Zealand Equine Research Foundation for providing financial support for my research and to Massey University for providing facilities and the doctoral scholarship, which enabled me to financially support myself.

I would like to thank my chief supervisor, Dr. Joanne Meers, for her friendship, support and enthusiasm throughout this project. I also thank my co-supervisors, Professor Colin Wilks and Dr. Richard Johnson for their time, advice, and encouragement.

I wish to acknowledge Dr. Brian Goulden, who helped me to establish links with the equine industry. Without him I would not have been able to organise horses for sampling. Thanks are also due to the horse owners who agreed to take part in the project and veterinarians who supplied some of the samples.

Thanks are due to people from the Centre for Equine Virology at the University of Melbourne, Australia. Special thanks to Professor M. J. Studdert, for providing me with the reference strains of EHV-2 and EHV-5, antiserum to equine adenovirus-1, and also for hosting me in his laboratory for a period of one month. This made it possible for me to test survey sera for the presence of antibodies to equine rhinoviruses. Many thanks to Nino Ficorilli for his technical assistance in getting through hundreds of serum samples within a short period of time. I also wish to thank Steven Holloway for his input into this work, particularly in designing primers to amplify the *gB* gene of EHV-5.

I am also grateful to all the staff members and fellow students at Massey University who helped me throughout the project. It is not possible to name everybody individually, but I particularly would like to express how much I appreciated the friendly, relaxed and stimulating atmosphere in the Department. I would especially like to thank my Polish friends, Magda and Jacek, for their friendship and support. Special thanks to Matthew for all the times we shared.

These acknowledgements wouldn't be complete without mentioning my horse, Travolta. He not only (unwillingly) provided leucocytes for the RDA experiment, but also has been a great companion for the last three years.

At last, by no means least, I would like to thank my mother for her love and support, and also for making it possible for me to come and study in New Zealand.

## Table of contents

---

Abstract.....	iii
Acknowledgements.....	v
Table of contents.....	vi
List of figures.....	x
List of tables.....	xii
Abbreviations.....	xiii
<b>CHAPTER 1:LITERATURE REVIEW .....</b>	<b>1</b>
1.1 EQUINE RESPIRATORY DISEASE .....	1
1.1.1 Respiratory disease as a cause of wastage for the equine industry.....	1
1.1.2 Equine respiratory disease – definition.....	2
1.2 INFECTIOUS AGENTS ASSOCIATED WITH EQUINE RESPIRATORY DISEASE.....	3
1.2.1 Viruses.....	3
1.2.2 Bacteria.....	7
1.2.3 Fungi.....	7
1.2.4 Other pathogens.....	7
1.3 EQUINE RESPIRATORY VIRUSES.....	8
1.3.1 Equine herpesviruses.....	8
1.3.2 Equine picornaviruses.....	27
1.3.3 Equine adenovirus.....	34
1.3.4 Equine arteritis virus.....	41
1.3.5 Equine Reoviruses.....	45
1.3.6 Equine parainfluenza virus-3.....	49
1.3.7 Equine influenza virus.....	52
1.4 AIMS AND SCOPE OF THE THESIS .....	53
<b>CHAPTER 2:DESIGN OF THE SURVEY .....</b>	<b>57</b>
2.1 INTRODUCTION .....	57
2.2 GENERAL MATERIALS AND METHODS .....	58
2.2.1 Horses.....	58
2.2.2 Collection of samples .....	64
2.2.3 Processing of samples .....	65
<b>CHAPTER 3:VIRUS ISOLATION.....</b>	<b>67</b>
3.1 INTRODUCTION .....	67
3.2 MATERIALS AND METHODS .....	68
3.2.1 Cell culture.....	68
3.2.2 Collection of samples .....	70
3.2.3 Processing of samples .....	70

3.2.4	<i>Virus detection</i> .....	71
3.2.5	<i>Polymerase Chain Reaction</i> .....	72
3.3	<b>RESULTS</b> .....	79
3.3.1	<i>Foals</i> .....	79
3.3.2	<i>Outbreaks</i> .....	82
3.3.3	<i>Yearlings</i> .....	83
3.3.4	<i>Other viruses</i> .....	83
3.3.5	<i>Isolates negative by PCR</i> .....	83
3.3.6	<i>Primary isolation</i> .....	84
3.3.7	<i>Comparison of cell culture and PCR results</i> .....	85
3.3.8	<i>Association with clinical signs</i> .....	86
3.4	<b>DISCUSSION</b> .....	87
3.4.1	<i>Lack of isolation of viruses other than herpesviruses</i> .....	87
3.4.2	<i>Unidentified viral isolates</i> .....	89
3.4.3	<i>Clinical significance of EHV-2 and EHV-5 infections</i> .....	90
3.5	<b>SUMMARY</b> .....	93
<b>CHAPTER 4:EHV-1/4 SEROLOGY</b> .....		<b>95</b>
4.1	<b>INTRODUCTION</b> .....	95
4.2	<b>MATERIALS AND METHODS</b> .....	96
4.2.1	<i>Serum neutralisation test (SN)</i> .....	96
4.2.2	<i>Blocking ELISA</i> .....	97
4.2.3	<i>Reproducibility of results</i> .....	98
4.2.4	<i>Definition of recent EHV-1 and EHV-4 infections</i> .....	98
4.3	<b>RESULTS</b> .....	99
4.3.1	<i>Reproducibility of results</i> .....	99
4.3.2	<i>Foals</i> .....	99
4.3.3	<i>Yearlings</i> .....	101
4.3.4	<i>Outbreaks</i> .....	105
4.3.5	<i>Comparison between ELISA and SN titres</i> .....	106
4.4	<b>DISCUSSION</b> .....	108
4.4.1	<i>Serology as a tool for diagnosis of recent EHV-1/4 infection</i> .....	108
4.4.2	<i>Time of EHV-1/4 infection</i> .....	113
4.4.3	<i>Protection and cross-protection from infection</i> .....	115
4.4.4	<i>Correlation between recent EHV-1/4 infection and presence of clinical signs</i> .....	116
4.5	<b>SUMMARY</b> .....	118
<b>CHAPTER 5:EQUINE RHINOVIRUS SEROLOGY</b> .....		<b>119</b>
5.1	<b>INTRODUCTION</b> .....	119
5.2	<b>MATERIALS AND METHODS</b> .....	120
5.2.1	<i>Serum neutralisation test</i> .....	120

5.3	RESULTS .....	121
5.3.1	<i>Equine Rhinovirus-1</i> .....	121
5.3.2	<i>Equine rhinoviru-2</i> .....	123
5.4	DISCUSSION .....	126
5.5	SUMMARY.....	131
<b>CHAPTER 6: EQUINE ADENOVIRUS SEROLOGY .....</b>		<b>133</b>
6.1	INTRODUCTION .....	133
6.2	MATERIALS AND METHODS .....	134
6.2.1	<i>Haemagglutination Inhibition test (HI)</i> .....	134
6.3	RESULTS .....	136
6.3.1	<i>Foals</i> .....	136
6.3.2	<i>Yearlings</i> .....	138
6.3.3	<i>Outbreaks</i> .....	139
6.4	DISCUSSION .....	140
6.5	SUMMARY.....	145
<b>CHAPTER 7: EQUINE ARTERITIS VIRUS, PARAINFLUENZA VIRUS-3 AND REOVIRUS-3 SEROLOGY .....</b>		<b>147</b>
7.1	INTRODUCTION .....	147
7.2	MATERIALS AND METHODS .....	148
7.2.1	<i>Equine arteritis virus</i> .....	148
7.2.2	<i>Parainfluenza virus-3</i> .....	148
7.2.3	<i>Mammalian Reovirus-3</i> .....	148
7.3	RESULTS .....	152
7.3.1	<i>Equine arteritis virus</i> .....	152
7.3.2	<i>Parainfluenza virus 3</i> .....	152
7.3.3	<i>Mammalian Reovirus 3</i> .....	152
7.4	DISCUSSION .....	153
<b>CHAPTER 8: THE SURVEY – GENERAL DISCUSSION .....</b>		<b>155</b>
8.1	INTRODUCTION .....	155
8.2	VIRUSES CIRCULATING IN NEW ZEALAND HORSES AND ASSOCIATION WITH CLINICAL SIGNS .....	156
8.2.1	<i>Outbreaks of respiratory disease</i> .....	156
8.2.2	<i>Yearlings from the yearling sales</i> .....	159
8.2.3	<i>Foals followed on a monthly basis</i> .....	160
8.3	TIME OF VIRAL INFECTIONS IN FOALS .....	161
8.4	SUMMARY.....	163
<b>CHAPTER 9: GENOMIC COMPARISON OF EHV-5 ISOLATES .....</b>		<b>169</b>
9.1	INTRODUCTION .....	169
9.2	MATERIALS AND METHODS .....	170



9.2.1	<i>Viruses</i> .....	170
9.2.2	<i>DNA extraction</i> .....	171
9.2.3	<i>Polymerase Chain Reaction</i> .....	171
9.2.4	<i>Cloning PCR products</i> .....	171
9.2.5	<i>Colony screening</i> .....	173
9.2.6	<i>RFLP of glycoprotein B gene</i> .....	173
9.2.7	<i>Sequencing and sequence comparison</i> .....	174
9.3	RESULTS .....	174
9.3.1	<i>EHV-5 isolated from different horses had different RFLP profiles</i> .....	175
9.3.2	<i>RFLP profiles of EHV-5 from the same horses were identical</i> .....	175
9.3.3	<i>Sequence comparison</i> .....	177
9.4	DISCUSSION .....	177
9.5	SUMMARY .....	183
<b>CHAPTER 10:REPRESENTATIONAL DIFFERENCE ANALYSIS OF EHV-2 INFECTED EQUINE LEUCOCYTES</b> .....		<b>185</b>
10.1	INTRODUCTION .....	185
10.1.1	<i>Approaches to investigation of differentially expressed genes</i> .....	186
10.2	MATERIALS AND METHODS .....	189
10.2.1	<i>Equine herpesvirus-2</i> .....	189
10.2.2	<i>RNA extraction</i> .....	190
10.2.3	<i>cDNA synthesis</i> .....	190
10.2.4	<i>Preparation of driver and tester</i> .....	191
10.2.5	<i>First round of amplification – generation of the first difference product (DP1)</i> .....	194
10.2.6	<i>Ligation of testers to J adaptors</i> .....	194
10.2.7	<i>Second round of amplification – generation of the second difference product (DP2)</i> .....	195
10.2.8	<i>Third round of amplification – generation of the third difference product (DP3)</i> .....	196
10.2.9	<i>Cloning of the DP3 amplicons</i> .....	196
10.2.10	<i>Dot blots</i> .....	197
10.2.11	<i>Preparation of probes</i> .....	197
10.2.12	<i>Hybridisation</i> .....	198
10.2.13	<i>Selection of clones for further analysis</i> .....	198
10.2.14	<i>Sequencing and molecular analysis</i> .....	199
10.3	RESULTS .....	199
10.4	DISCUSSION .....	203
10.5	SUMMARY .....	209
<b>CHAPTER 11:CONCLUDING REMARKS</b> .....		<b>211</b>
<b>APPENDICES</b> .....		<b>215</b>
<b>BIBLIOGRAPHY</b> .....		<b>237</b>

## List of figures

<b>Figure 3.1:</b> Results of EHV-1/4 PCR visualised on a 1.5% EtBr stained gel and a corresponding dot blot probed with either EHV-1 or EHV-4 probe as indicated.....	80
<b>Figure 3.2:</b> Viruses isolated from foals followed on a monthly basis: group A (A), group B (B), and group C (C).....	81
<b>Figure 3.3:</b> An example of amplification products from a PCR with EHV-2 (upper part of a gel – samples 1' to 16') and EHV-5 (lower part of a gel – samples 1 to 16) primers, visualised on 1.5% EtBr stained gel (a) and a corresponding dot blot probed with either EHV-2 probe (b) or EHV-5 probe (c).....	85
<b>Figure 4.1:</b> Foals group A: EHV-1/4 SN titres (A) and EHV-1 ELISA titres (B).....	100
<b>Figure 4.2:</b> Foals group B: EHV-1/4 SN titres (A) and EHV-1 ELISA titres (B).....	101
<b>Figure 4.3:</b> Foals group C: EHV-1/4 SN titres (A) and EHV-1 ELISA titres (B).....	102
<b>Figure 4.4:</b> Yearlings: EHV-1/4 serology: SN titres (A) and blocking ELISA titres (B). ....	104
<b>Figure 4.5:</b> Outbreaks: EHV-1 SN titres (A) and ELISA titres (B).....	106
<b>Figure 4.6:</b> Comparison between EHV -1 blocking values and SN titres of sera from foals (A), yearlings from the yearling sales (B), and horses from outbreaks of respiratory disease (C).....	107
<b>Figure 5.1:</b> Foals group A: ERhV-1 serology .....	122
<b>Figure 5.2:</b> Foals group A: ERhV-2 serology.....	124
<b>Figure 5.3:</b> Foals group B: ERhV-2 serology.....	125
<b>Figure 5.4:</b> Foals group C: ERhV-2 serology.....	125
<b>Figure 5.5:</b> Outbreaks: ERhV-2 serology. ....	126
<b>Figure 5.6:</b> Yearlings: ERhV-2 serology. ....	126
<b>Figure 6.1:</b> Foals group A: EAdV-1 serology.....	136
<b>Figure 6.2:</b> Foals group B: EAdV-1 serology.....	137
<b>Figure 6.3:</b> Foals group C: EAdV-1 serology.....	138
<b>Figure 6.4:</b> Yearlings: EAdV-1 serology.....	139
<b>Figure 6.5:</b> Outbreaks: EAdV-1 serology.....	139
<b>Figure 8.1:</b> The activity of equine respiratory viruses in horses and foals from outbreaks of respiratory disease. ....	157
<b>Figure 8.2:</b> Association between the presence of clinical signs and recent viral infections in yearlings from the yearling sales. ....	159
<b>Figure 8.3:</b> Foals group A (A), B (B) and C (C): mean titres to EHV-1/4 (i), EAdV-1 (ii), ERhV-2 (iii), ERhV-1 (iv), and virus isolation from nasal swabs (v).....	165
<b>Figure 9.1:</b> Amplification products of PCR with primers (rp + fp) specific for EHV-5 <i>gB</i> . ....	174
<b>Figure 9.2:</b> Amplification products of PCR colony screening with EHV-5 rp and T7 primer. ....	174
<b>Figure 9.3:</b> The position of predicted <i>Bfa</i> 1 sites in a cloned EHV-5 rp-T7 primer amplification product (yellow) and in a corresponding fragment of EHV-2 sequence (white).. ....	175
<b>Figure 9.4:</b> <i>Bfa</i> 1 digest of EHV-5 rp-T7 primer amplification products.....	176
<b>Figure 9.5:</b> Comparison of the nucleotide sequences of the EHV-5 inserts from the clones listed on the left.....	178
<b>Figure 9.6:</b> Comparison of the predicted amino acid sequences from the N terminus of <i>gB</i> . ....	179
<b>Figure 10.1:</b> Schematic diagram of cDNA RDA (adapted from Frazer <i>et al.</i> 1997).....	191
<b>Figure 10.2:</b> Approximately 0.5 – 1 µg of R representations (A), DP1 (B), DP2 (C), and DP3 (D) run on 1% agarose gels and stained with gel star nucleic acid stain (FMC). ....	200
<b>Figure 10.3:</b> Colony dot blots of DP3(-) or DP3(+) products, probed with either DP3(+) or DP3(-) probes, as indicated.....	201

**Figure 10.4:** Re-amplification products of PCR using selected DP3(-) amplicons (top) and DP3(+) amplicons (bottom) as target DNA. .... 202

**Figure 10.5:** Approximately 500 ng of R representations, DP1, DP2, and DP3 products, obtained after subtraction of either (+) or (-) samples, probed with DIG-labelled probes, as indicated. .... 203

List of tables

---

Table 2.1: Foals sampled on a monthly basis.....	59
Table 2.2: Yearlings from the yearling sales 1997.....	61
Table 2.3: Horses from outbreak TA.....	63
Table 3.1: PCR primers and programs used in the study.....	74
Table 3.2: EHV-5 isolation from foals. ....	80
Table 3.3: Viruses isolated from horses from outbreaks of respiratory disease .....	82
Table 3.4: Viruses isolated from yearlings from the sales.....	83
Table 3.5: Isolates negative by PCR.....	84
Table 3.6: Herpesvirus (EHV-2, EHV-5, or both) isolation from horses from outbreaks of respiratory disease and yearlings from the yearling sales.....	86
Table 5.1: Outbreaks of respiratory disease - horses positive for ERhV-1 antibodies .....	123
Table 7.1: Haemolysis standards .....	151
Table 7.2: Haemolysis allowances for complement controls .....	151
Table 9.1: Details of the source of the EHV-5 isolates used in the study.....	170
Table 9.2: Comparison of RFLP patterns obtained for different EHV-5 isolates.....	176
Table 10.1: GenBank sequences with significant homology to the listed clones .....	202

# Abbreviations

---

AGID	agar gel immunodiffusion
AIDS	acquired immunodeficiency syndrome
ATP	adenosine-5'-triphosphate
ATV	Antibiotic / trypsin / versene
bp	base pair
BLAST	basic local alignment search tool
BSA	bovine serum albumin
CF	complement fixation
Con A	concanavalin A
COPD	chronic obstructive pulmonary disease
CPE	cytopathic effect
CTL	cytotoxic T lymphocyte
CV	coefficient of variation
DD	differential display
ddNTP	dideoxynucleoside-5'-triphosphate
DIG	digoxigenin
dNTP	deoxynucleoside-5'-triphosphate
DP	difference product
EAdV	equine adenovirus
EAV	equine arteritis virus
EBV	Epstein-Barr virus
EDTA	ethylenediamine tetra-acetic acid
EFK	equine foetal kidney
EHV	equine herpesvirus
ELH	Earles lactoalbumin hydrolysate
ELISA	enzyme linked immunosorbent assay
EM	electron microscopy
ERhV	equine rhinovirus
F	fusion (glycoprotein)
FBS	foetal bovine serum
FMDV	food-and-mouth disease virus
fp	forward primer
gB	glycoprotein B
GM	growth medium

GPCR	G-protein-coupled receptor
HA	haemagglutination
H & E	haematoxinilin and eosin
HCMV	human cytomegalovirus
HI	haemagglutination inhibition
HIV	human immunodeficiency virus
HN	haemagglutinin-neuraminidase
HSV	herpes simplex virus
HVS	herpesvirus saimiri
IAA	isoamyl alkohol
Ig	immunoglobulin
IL	interleukin
IPTG	isopropylthio- $\beta$ -D-galactoside
kbp	kilobase pairs
LAT	latency associated transcript
LB	Luria-Bertolini broth
MAb	monoclonal antibody
MC	mononuclear cells
MCP	monocyte chemoattractant protein
MEM + n	minimal essential medium + non-essential amino acids
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
MM	maintenance medium
NK	natural killer
O.D.	optical density
OPD	ortho-phenylenediamine dihydrochloride
ORF	open reading frame(s)
PBL	peripheral blood leucocyte(s)
PBS	phosphate buffered saline, pH 7.0
PCR	polymerase chain reaction
PHA	phytohaemagglutinin
PI-3	parainfluenza virus-3
PNK	polynucleotide kinase
PSK	penicillin / streptomycin / kanamycin
RANTES	regulated on activation of normal T cell expressed and secreted

RBC	red blood cell(s)
RDA	representational difference analysis
Reo	mammalian reovirus
RFLP	restriction fragment length polymorphism
RK-13	rabbit kidney-13
rp	reverse primer
RT	room temperature
RT-PCR	reverse transcriptase-PCR
SDS	sodium dodecyl sulphate
SN	serum neutralisation
SPF	specific pathogen free
SV40	simian virus 40
TAE	tris / acetate / EDTA
TBE	tris / borate / EDTA
TCID <sub>50</sub>	tissue culture infective dose 50%
TE	tris / EDTA
TGF	transforming growth factor
Th1	CD4+ T helper type 1 lymphocyte
TK	thymidine kinase
TNF	tumor necrosis factor
SN	serum neutralisation
Vero	African green monkey (cells)