Equine Respiratory Viruses in New Zealand

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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.

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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'-triphospate
DIG	digoxigenin
dNTP	deoxynucleoside-5'-triphospate
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 hydrolisate
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	haematoxilin 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-β-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
$TCID_{50}$	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)