Equine respiratory viruses in New Zealand

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Abstract

Equine respiratory disease has been recognised as an important cause of wastage resulting in financial loss for the equine industry worldwide. Limited studies have been conducted on equine respiratory viruses in New Zealand, particularly within the past ten years. As such, the objective of the present study was to determine 1) which respiratory viruses circulate among horses from selected New Zealand locations and 2) whether or not infection with any of the viruses identified was associated with clinical disease. A survey was conducted on 85 horses to detect the presence of viruses known to be associated with equine respiratory disease. Nasal swabs were taken from 52 horses with signs of respiratory disease and from 33 healthy horses. Horses were sampled from within the Manawatu and Hawkes Bay regions by convenience.

Species specific PCR was performed directly on nasal swabs. The only viruses detected were equine herpesviruses (EHV) types 1, 2, 4 and 5. Of the 52 horses with respiratory disease, 3 tested positive for EHV-1, 14 for EHV-4, 23 for EHV-2 and 26 tested positive for EHV-5. Of the 33 healthy horses 2 tested positive for EHV-2, one of which also tested positive for EHV-5. Over all, the detection of herpesviruses was significantly associated with respiratory disease (p value <0.0001). Detection of individual virus species (EHV-2, EHV-4 or EHV-5) was also significantly associated with respiratory disease (p value 0.0002, 0.0006, <0.0001, respectively). The sample size was not large enough to evaluate the significance of EHV-1 detection and respiratory disease.

Virus isolation performed on the samples from the 52 horses with respiratory disease detected EHV types 1, 2, 4 and 5. No viruses were detected from the 33 samples of healthy horses. There was poor correlation between virus isolation and PCR results, particularly with regard to EHV-4.

This work gives a recent contribution to the knowledge of equine respiratory viruses in New Zealand. Although the sampling was performed by convenience, the results suggest an association between equine herpesviruses types 2, 4 and 5 and equine respiratory disease.

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List of publications

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Dunowska M, McBrearty KJ, Biggs PJ, Murray A. Survey of equine respiratory viruses in New Zealand. IX International Congress of Veterinary Virology 4-7 Sep 2012, Madrid, Spain.

McBrearty K and Dunowska M. Equine respiratory viruses in New Zealand (2009). In: *5th Australasian Virology Society Meeting* 13-17 Dec 2009, Lorne, Victoria, Australia.

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Sciences); Lanes 1-15: CPE positive cell lysates; Lane 16: EHV-1/4 positive control; Lane 17: Negative control (equine DNA). Expected products: EHV-1 649bp, EHV-4 507bp. Cell lysates 1-5, 11, 12 &14 are positive for EHV-4 and none are positive for EHV-1 (note bands 2, 11 & 14 are very weak, but are clearly positive on the dot blot). Dots with less or the same intensity as the negative control were considered negative. 54

Abbreviations

7TMR	Seven trans-membrane receptor
Ab	Antibody
bp	Base pair
CF	Complement fixation
CFU	Colony forming units
CPE	Cytopathic effect
CTL	Cytotoxic T-lymphocyte
DIG	Digoxigenin
DMEM	Dulbecco’s modified eagle medium
DNTPs	Deoxynucleoside-5’ –triphosphate
EAdV	Equine Adenovirus
EAV	Equine arteritis virus
EDTA	Ethylenediamine tetra-acetic acid
EFK	Equine fetal kidney (cells)
EHV	Equine herpesvirus
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscope
ERAV	Equine rhinitis A-virus
ERBV	Equine rhinitis B-virus
EtBr	Ethidium bromide
FBS	Fetal bovine serum
FMDV	Foot and mouth disease virus

gB	Glycoprotein B
GM	Growth medium
IAD	Inflammatory airway disease
Ig	Immunoglobulin
IL	Interleukin
INF	Interferon
LRT	Lower respiratory tract
MHC	Major histocompatibility complex
MM	Maintenance media
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
RK-13	Rabbit kidney -13 (cells)
RT-PCR	Reverse transcriptase -PCR
SDS	Sodium dodecyl sulphate
VERO	African green monkey (cells)
VN	Virus neutralization