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

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Abstract

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 foetuses 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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Abbreviations

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

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
$TCID_{50}$	Tissue culture infective doses 50%
TK	Thymidine kinase
VN	Virus neutralisation