Latent Equine Herpesvirus Infections in Horses

This thesis is presented for the degree of Doctor of Philosophy at Murdoch University

by

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I declare that this thesis is my own account of my research and contains work which has not previously been submitted for a degree at any tertiary education institution

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Abstract

A significant characteristic of the herpesviruses is that they form latent infections in infected hosts, and can be reactivated to again induce lytic infections by stressors. This thesis deals with an epidemiological investigation of equine herpesvirus infection, particularly gammaherpesvirus infections, in foals and if there was evidence of reactivation of latent virus infections by stressors such as those associated with weaning.

A longitudinal study of EHV infections in young foals and the effect of stressors such as weaning on the prevalence of virus infection was undertaken by the detection of DNA and mRNA of EHV2, EHV5, EHV1 and EHV4 in peripheral blood leukocyte (PBL) and nasal swabs (NS) from 13 mares and 46 foals in 4 stables. EHV2 and EHV5 infections were detected commonly in the study population but infections with the alphaherpesviruses EHV1 and EHV4 were not detected, although lytic infections by the alphaherpesviruses may have been missed due to the frequency of sampling. Age differences in the prevalence of EHV2 and EHV5 infection were detected: the prevalence of EHV2 was higher in young foals than in older foals and adult animals; the prevalence of EHV5 was higher in older foals and adults than in younger foals. The prevalence of EHV2 and EHV5 infection increased in association with weaning, presumably in association with stressors associated with weaning, but was not clearly associated with disease in the weaned animals. It was also observed that EHV5 produced a transient lytic infection in PBL of young foals but tended to produce a persistent lytic infection of PBL in older foals and adults. Persistent lytic EHV5 infections of ≥37 weeks duration were also detected in 2 of 13 adult mares and this has not been reported previously. The persistent lytic infection of PBL was not associated

with the detection of virus in NS and the mares with persistent lytic infection of PBL with EHV5 did not transfer the infection to their foals.

To determine if any of the animals examined were latently infected with the alphaherpesviruses, an examination for transcripts of genes 63 and 64 of EHV1 and EHV4, putative latency-associated transcripts (LAT) of EHV, was undertaken. Evidence of these transcripts was detected in PBL and bronchiolar lymph nodes in the absence of transcripts of the structural gB, supporting previous studies indicating that transcripts of genes 63 and 64 may represent LAT. In PBL, EHV1 gene 64 RNA transcripts but not gene 63 transcripts were detected in PBL. In bronchiolar lymph nodes, EHV1 gene 64 (but not gene 63) RNA transcripts were detected. In contrast, EHV4 infection was detected in the trigeminal ganglia only and there was no evidence of EHV4 infection in lymph nodes or PBL. In the trigeminal ganglion, EHV4 gB DNA and gene 63 RNA transcripts were detected. The presence of RNA transcripts of EHV1 gene 64 in PBL and lymph node in the absence of any evidence of the replication of structural proteins suggests PBL and lymph node are sites of EHV1 latent infections. The presence of EHV4 gene 63 transcripts in trigeminal ganglia in the absence of any evidence of replication of structural proteins suggests the trigeminal ganglion is the major site of latency of EHV4.

As a potential means of detecting latency of the gammaherpesvirus EHV2, 4 EHV2 genes ORF74, E4, E8 and E10 were selected as having possible roles during EHV2 latency based on sequence analysis and comparison with gene products identified or postulated as having roles in latency in other gammaherpesviruses. Kinetic transcription of these genes was evaluated in an *in vitro* time course study using a non neuronal cell line (equine kidney [EK] cells). While the gB and gH genes encoding structural glycoproteins were abundantly transcribed *in vitro,* the 4 putative EHV2

latency-associated genes were minimally transcribed during lytic infection in EK cells, a result analogous to results obtained for the expression of LATs in other gammaherpesviruses. Attempts to demonstrate transcription products of these genes in PBL or other tissues of horsed presumed to be latently infected with EHV2 (in which gB transcripts had been detected previously) and actively infected with EHV2 (in which gB transcripts had been detected at the time of sampling), were unsuccessful.

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