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Effect of Time of Day of Vaccination on IL-10 Gene Expression in Horses
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INTRODUCTION
• Previous studies have shown that circadian rhythms regulate molecules of the immune system, such as cytokines [1].
• Cytokines provide intracellular communication for immune cells and help regulate inflammation and immune responses [2,3].
• Interleukin-10 (IL-10) is an anti-inflammatory cytokine, and inhibits antigen presentation as well as production of pro-inflammatory cytokines (Figure 1) [4,5].
• IL-10 is expressed in response to an activation stimulus such as over production of inflammatory factors or the presence of antigens [6].
• Research has shown that IL-10 production peaks around 2200 h, which suggests a bias towards adaptive immunity during the night and early morning [6].
• Vaccines stimulate the adaptive immune response so the immune system can react faster and more strongly when the antigen is encountered again [8].
• Although it has been suggested that time of day affects immune response, few studies have evaluated IL-10 gene expression to determine if time of day of vaccination does influence immune responses [3].

OBJECTIVE
• The objective of this study was to determine if time of day of vaccination can influence the expression of IL-10, an anti-inflammatory cytokine, in horses.

METHODS
• Eight American Quarter Horse mares (10.5 ± 5.8 yrs) and six American Miniature Horse geldings (7 ± 2.6 yrs) were randomly assigned to an AM or PM vaccination group.
• All horses had access to water and mixed-grass hay ad libitum, and were housed in outside paddocks with access to shelter to shelter at all times.
• Horses in the AM and PM vaccination groups were vaccinated intramuscularly against Eastern and Western encephalomyelitis, rhinopneumonitis (EHV-1 and EHV-4), influenza (type A2), and West Nile Virus at 0700 or 1900 h.
• Blood samples were collected in PAXgene blood tubes via jugular venipuncture immediately prior to vaccination (d 0) and then on d 7, 14 and 21 post-vaccination at 1200 h.
• RNA was extracted using a PAXgene Blood RNA Kit. Reverse transcription reactions were performed using the SuperScript IV protocol to produce cDNA.
• Quantitative real-time PCR reactions were carried out using the AgPath-ID One Step RT-PCR Kit on an ABI Tagman 7500 platform.
• Relative quantification of fold change was calculated using the 2-ΔΔCT procedure and normalized to the housekeeping gene (BGUS).

RESULTS

DISCUSSION
• IL-10 has previously been shown to decrease antigen presentation and inhibit pro-inflammatory cytokines [2,3].
• Higher IL-10 expression in the AM vaccinated group suggests a greater immune response in horses when vaccinated in the morning compared to the evening hours.
• Increased IL-10 expression in the AM vaccinated group may be mediated by melatonin, which is produced at night and has been shown to modulate cytokine production [7].
• Blood samples taken on d 0 were taken immediately prior to vaccination, in contrast to post-vaccination samples which were taken at 1200 h.

CONCLUSION
• The data shown here suggest that vaccinations administered in the morning elicit a stronger immune response. However, additional studies are needed to confirm these findings.

REFERENCES