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## SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY FOR SERUM AMYLOID A PROTEIN IN HORSES

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Serum Amyloid A (SAA), a putative serum precursor of the Amyloid A (AA) protein which constitutes amyloid fibrils in secondary amyloidosis, has been clinically evaluated as one of the sensitive acute-phase reactants in horse serum. The SAA concentration has been analyzed by single radial immunodiffusion (SRID). Although the SRID method is very simple, it is time-consuming and requires not only antigen-antibody binding but also the formation of an insoluble complex. In this study, an attempt to develop a sensitive and highly reproducible sandwich ELISA method for measurement of SAA in horse serum was made. The reproducibility of this assay was found to be acceptable because the covariant values (CVs) of the assay were less than 15%. Correlation analysis between the present method and the SRID test showed a correlation coefficient ( $r$ ) of 0.95, which confirmed the accuracy of the ELISA method.

The amino acid composition of purified horse SAA was also analyzed. From the analysis, it was suggested that horse SAA was similar to human SAA.

The equine SAA concentration was measured with this assay. In clinically normal horses, the concentration of SAA was relatively high from immediately after birth to 2 weeks of age. After this the concentration showed periodic fluctuations in the range of approximately 10 to 20  $\mu\text{g/ml}$ .

The mean ( $\pm$ SD) concentration of SAA in foals ( $\leq 12$  months old) and in adult horses ( $\geq 18$  months old) were  $21.23 \pm 12.20$  and  $14.93 \pm 9.07$   $\mu\text{g/ml}$ , respectively.

In mares during the perinatal period, the SAA concentration remained stable and within the normal range up to 4 months before parturition. After delivery, it increased quickly and reached a peak value of  $101.29 \pm 98.82$   $\mu\text{g/ml}$  on day 3 postpartum, and then began to decrease at 2 weeks postpartum, returning to within the normal range by 1 month postpartum. In the horses with experimentally induced inflammation, the SAA concentration increased quickly and reached the highest value, approximately 4 to 40 times higher than pre-treatment values, on day 2 after treatment. It then returned to the baseline within 2 to 4 weeks in association with the disappearance of local inflammatory signs. The SAA concentration was high in most horses with clinical signs of inflammation.

It was concluded from these data that equine SAA is a sensitive acute-phase reactive protein that increases in the early phase of various acute inflammations. The ELISA method was proved to be a sensitive and reliable assay for measuring SAA in horse serum.