

ABSTRACT #314

DEVELOPMENT OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SERUM VITAMIN B6 ASSAY IN CANINES WITH CALCIUM OXALATE UROLITHIASIS. J Clemans, P Imerman, J Phillips, K Deitz, A Jergens. Iowa State University College of Veterinary Medicine, Ames, IA.

Calcium oxalate urolithiasis now represent the second most common canine urolith in North America. As in people, these stones have a high rate of recurrence with a suspect genetic predisposition. The risk factors for these stones are poorly understood and current medical management does not preclude recurrence. Vitamin B6 deficiency is a risk factor in humans but this has not been evaluated in the canine population. The goal of the study was to develop an assay to detect Pyridoxyl-5-Phosphate (PLP) (active form of vitamin B6) in canine serum and assess dogs with and without calcium oxalate stones. A preliminary study of vitamin levels in predisposed versus non-predisposed breeds was also performed.

A High Performance Liquid Chromatography (HPLC) assay was developed using pooled canine serum from the ISU Veterinary Medical Center clinical pathology laboratory. Serum from dogs with stones and without stones were light protected and stored at -80°F within 12 hours of collection. Serum concentration of PLP was obtained using a standard curve with known concentrations of research grade PLP in water for each run. Recovery was also measured for each run using a known concentration of PLP in serum. Runs with recoveries lower than 80% were not allowed. For initial assessment for differences in serum concentration, dogs were separated into CaOx group (3 dogs) and non-CaOx group (4 dogs).

Vitamin B6 concentrations in canine serum were unstable at 2^o and -15^oC. Storage at these temperatures caused a rapid loss of 20% in two days and 50% at 14 days respectively. When stored at -80^oC for 14 days, approximately 100% remained. Freezing and thawing the samples resulted in minimal recovery loss after three cycles. The pilot study revealed that the dogs with calcium oxalate urolithiasis were significantly lower (p value <0.05) in vitamin B6 concentrations than those without stones, with mean concentrations of 29 ppb (SD=18) and 123 ppb (SD=70) respectively. Preliminary results comparing serum concentrations of dogs of a predisposed breed (mean levels 55 ppb) to dogs not of predisposed breeds support a lower vitamin B6 level in predisposed breeds.

An HPLC assay was successfully utilized to reliably measure serum vitamin B6 levels in canine serum and a deficiency relative to dogs without urinary stones was present in calcium oxalate forming dogs. A difference in vitamin concentrations was supported in an evaluation of a breed predisposed to these stones and dogs not predisposed. Vitamin B6 deficiency may prove to be a risk factor for calcium oxalate urolithiasis and further evaluation is currently underway.