INTRODUCTION:
The canine cruciate ligament (CL) complex is comprised of the anterior (ACL) and posterior cruciate ligaments (PCL). CLs consist of sparse fibroblasts surrounded by dense collagenous matrix. Degeneration of the ACL in the most common disease of the canine femorotibial joint. Characterisation of cell and matrix changes such as loss of collagen architecture and chondrocytic transformation of cells allows CLs to be categorised on a scale from 0 (no change) to 3 (severe changes), (Vasseur, Pool et al. 1985).

Elastin has traditionally been considered a minor component of the extracellular matrix (ECM) of ligament tissue. Elastin fibres (EFs) comprise an amorphous elastin core within a fibrillin-containing microfibril (MF) scaffold and impart extensibility to soft tissue. Formation of the EF is a highly regulated pericellular process in which tropoelastin is transferred to a MF scaffold and stabilised through formation of the elastin-specific crosslinks. Quantitative data on the level of in CLs is extremely limited. Collectively, MFs and EFs are known as elastic fibres. Failure of elastic fibres has been implicated in a number of serious diseases. In this study, we aim to quantify elastin biochemically in canine CLs. We then aim to compare the biochemical results with detailed histological analysis and elastic fibre distribution.

MATERIALS AND METHODS:
Elastin fibres were harvested from seven skeletally mature Greyhound with no macroscopic evidence of femorotibial pathology. Tissue from proximal, middle and distal sections of each CL was provided for histology and biochemical analysis. Sequential sections of 4µm from paraffin-embedded samples were stained with haematoxylin and eosin (H&E), Verhoeff’s iodine-iron haematoxylin (EVH) to show only EFs and elastin van Gieson’s stain (or Miller’s stain, M) to show both EFs and MFs. Only EFs stained with the 1,9-dimethylene blue dye binding assay. Elastin, hydroxyproline assay. Total sulphated glycosaminoglycan (sGAG) was assessed using the 1,9-dimethylene blue dye binding assay (Biocolor Ltd, N. Ireland). Collagen content was determined by PicroSirius assay (Vasseur et al. 1985). The broad grade 1 category was subdivided with a more detailed novel scoring system, allowing all samples to be ranked according to degree of matrix change. All GH samples were graded 0-3 according to criteria previously described (Vasseur et al. 1985). The broad grade 1 category was subdivided with a more detailed novel scoring system, allowing a score from 0-32 to be awarded. These results are referred to as modified Vasseur Score (mVS). A novel scoring system was also developed to quantify changes in MF staining. EVH and M stained sections were examined by one blinded assessor (KDS) for differences in MF staining for five criteria: interfascicular and interbundle regions, ligament substance (intrabundle), and the extent and degree of pericellular staining. These results are referred to as Miller’s Score (MS).

RESULTS:
Histology: As all the CLs in this study were assigned grade 1 according to the Vasseur scale, they could be additionally scored on our novel scoring system allowing all samples to be ranked according to degree of CL matrix change. CLs graded with the modified scoring system (mVS), had an average score of 11.9 ± 3.3 (range 5.5-21.5, median 12). There were no significant differences in mVS within or between ligaments. Where there was loss of collagen architecture a reduction in EFs was noted. In contrast, tissue with more advanced matrix change showed a marked increase in MF quantity, with changes in distribution. Cells that had undergone chondrocytic change commonly had increased pericellular MF staining. Furthermore, increased MF staining was noted in a subgroup of samples. The ligament substance in all CLs with advanced matrix change. If there was loss of collagen architecture, then the defect commonly showed increased MF staining with much less regular organisation.

Higher M scores were seen in the ACL (5.5 ± 2.0) than the PCL (4.3 ± 1.9), but were not statistically significantly different. The mid ACL had significantly lower MS than the mid PCL (p=0.039), proximal ACL (p=0.023) and distal ACL (p=0.001). The scoring system for the Miller’s staining showed a strong correlation with the mVS (0.690, p=0.000), confirming the histological observations.

Biochemistry: The average value of Elastin in the ACL was 9.86 ± 3.97 % dry weight ligament and 10.79 ± 4.37 in the PCL, but this difference was not statistically significant (p=0.28). Despite this large range of elastin values from all CLs, pairs of CLs had very similar elastin content. There was no significant variation in elastin content according to location (proximal, middle or distal), age, sGAG or % dry matter. No correlations were noted between elastin and mVS (r = -0.016, P-Value = 0.897) and MS (r = -0.104, P-Value = 0.404) in the whole or ligament subsections. Collagen as a percentage of dry weight averaged 75.34% ± 5.19% (range 62.2% - 86.7%). Water content in the ACL was 59.3 ± 3.1 % and 61.9 ± 2.2% in the PCL. The ACL had a significantly lower water content than the PCL (p=0.002). Furthermore, water content did vary significantly according to location within ligaments (p=0.035). Distal CLs had significantly lower water content than the respective middle ligaments, and the middle ACL had a significantly lower water content than middle PCL (p=0.007). The mean sGAG content was 0.062% ± 0.002% (p=0.001). Significantly greater sGAG was noted in the ACL than the PCL. In the ACL significantly greater sGAG content was found distally, whereas in the PCL significantly greater sGAG was found in the middle region. Significant correlation with mVS (0.389, p=0.002) and MS (0.543, p<0.000) was also observed.

DISCUSSION:
In this study we have shown that although elastin fibres appear to decrease with increased mVS or CL matrix changes, a marked production of MFs as matrix change progresses. Through the development of novel histological scoring systems, we suggest the increase in MFs to be proportional to the degree of matrix change. As far as we are aware, this has not been described in any ligament. The lack of correlation between elastin content and MS in the dog would appear to suggest the MF production is not associated with either elastin production or subsequent incorporation into EFs, thus is entirely fibrillin driven. MFs may have a number of roles in the CL complex including provision or maintenance of elasticity, stabilization of blood vessels, anchoring tissue or guidance of cell migration. It may be the increased quantity of OFs observed in the dog reflects a healing response. The lack of EFs in areas of mineralization would suggest that as matrix change progresses, there would be an overall loss of EFs. As EFs are not produced in adult tissue, these changes may reflect an irreversible loss, and may have significant implications for ligament pathogenesis. Elastin has been suggested to have a mechanical role in the CL complex. High elastin content in some ligaments did not correspond to increased numbers of EFs, and bore no relationship to MF production. It would therefore seem plausible that the variation in CL elastin observed between dogs may have non-mechanical functions. Although our study lacked samples of healthy and of high grade degenerative tissue, the absolute lack of any relationship with mVS and elastin through grade 1 matrix changes suggests elastin does not play a significant role in the progression of matrix change once initiated. The elastin assay used was unable to distinguish between insoluble and degraded elastin peptides trapped within the matrix. Matrix changes within the canine CLs are associated with increases in elastolytic enzymes such as cathepsin K and MMP-2 and -9. Whether the high elastin content seen in some pairs of CLs relative to others arises from increased transcription, increased degradation of EFs or from failure to remove degraded peptides remains to be elucidated.

REFERENCES:

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