Changes in articular cartilage in relation to subchondral bone remodeling in race horses. 
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ABSTRACT INTRODUCTION:
Osteoarthritis (OA) is a disease involving all the joint tissues. There is a persisting debate as to where the earliest changes of OA occur, cartilage or bone. Subchondral bone (SCB) remodeling is a common finding with cartilage lesions in both equine and human joints [1,2]. Focal SCB porosity in association with articular cartilage (AC) damage in the third metacarpal/tarsal bone has been previously reported in horses [3,4,5]. The aim of the present study was to characterize lesions in both cartilage and SCB of the third carpal bone (C3) in various stages of naturally occurring equine OA to attempt to elucidate the early events in the disease.

METHODS:
Specimen collection: Fifteen C3 were collected post mortem from racing Standardbreds. The C3 were assessed for cartilage macroscopic lesions and classified into 3 groups: Control (CO) group (no lesions), early osteoarthritis (EOA) group (fissures, partial thickness erosions in an area ≤ 100 mm²), advanced osteoarthritis (AOA) group (partial to full thickness erosions in an area ≥ 100mm²) (Fig. 1). Horses had a mean age of 6.6 ± 1.6 years in the CO group (n = 5), 5.6 ± 2 years in the EOA (n = 5) group and 5 ± 2.1 years in the AOA group (n = 5).
Core Harvest: Two osteochondral plugs of 1 cm in diameter (Figure 1) were harvested from a dorsal site where focal OA lesions characteristically arise (core 1) and in a more palmar remote site (core 2) where lesions are rarely encountered.

Micro-CT Protocol: The cores were scanned using a Micro CT Scanner. A subjective visual assessment was first used to grade micro-CT scans in 2D reconstruction (slice with most severe lesions evident). Then 3 regions of interest (ROI) were scanned in the C group (Figure 2) without any pits in the SCB surface. Five regions of interest (ROI) were defined for each sample in all groups that had a pit in the SCB (Figure 2). Parameters assessed included: Bone mineral density (BMD); Bone Volume Fraction (BV/TV); Trabecular thickness (Tb.Th); Trabecular spacing (Tb.Sp); Trabecular number (Tb.N); Fragmentation Index (Tb.Pf); Degree of anisotropy (Da); Structure Model Index (SMI).

Histology: Demineralized sections were made at a site corresponding to the Micro CT images with the worst structural damage in the core and were stained with Safranin O-Fast Green (SOFG) (Figure 3). Sections were graded semi-quantitatively by 2 blinded observers. Slides were assessed for cartilage degenerative changes with an adapted modified Mankin modified grading system. Microcracks in the calcified cartilage and pits in the SCB were also quantified.

RESULTS:
Micro-CT visual assessment: Micro CT visual assessment score was significantly higher in core 1 from the AOA group, but not the EOA group, when compared with the CO group (p = 0.006). An area of focal porosity was typically present deep to the pits in the articular surface when present. No significant difference was identified between cores 2 in any of the groups, highlighting the focal nature of the disease in the dorsal aspect of C3. The SCB was sometimes highly remodelled even if few or moderate changes were present at the AC surface.

Micro CT quantitative analysis: In the AOA group there was a statistically significant decrease in BMD, BV/TV and Tb.Sp in ROI L compared with ROI R. A significant increase of Tb.Th was also noticed in ROI L compared with ROI R (Figure 2). The comparison of the mean values of ROI L and C between core 1 and 2 revealed a significant increase for the BMD and BV/TV in OA (AOA + EOA) group. The Tb.Th of core 2 was significantly increased in the AOA group and the Tb.Sp was decreased in core 2 in the AOA group in comparison to core 1.

Histological analysis: An increase in histological score was observed with the progression of macroscopically evident cartilage changes. Cartilage fissures, erosions and a decrease in chondrocytes were the principal lesions encountered. The histological examination also revealed the presence of cracks in the calcified cartilage that significantly increased in number in OA (EOA + AOA) (Figure 4) when compared with the control group (p = 0.04). There was a positive and significant correlation between the histological score and the Micro CT visual assessment (r = 0.66, P<0.01) in the cores. The latter were significantly increased in AOA groups compared with the CO group.

DISCUSSION: This study illustrates that OA involves a degenerative process of both the articular cartilage and underlying SCB in this naturally occurring model of repetitive trauma induced OA in horses. In experimental models of repetitive trauma calcified cartilage cracks have also been observed by others [6]. Microcracks in the CC have also been reported as an early sign of articular failure in Thoroughbred horses but in a different joint, highlighting that it is a common finding in this disease process [4,5,7]. Furthermore SCB remodeling has also been observed in advanced osteoarthritis in people [2]. These findings illustrate a need to not only treat cartilage surface injury in OA but concomitantly address the underlying major structural alterations in the bone to return the cartilage bone unit to health. It also suggests that arthroscopic evaluation (equivalent to the macroscopic evaluation performed here) of the articular cartilage surface does not permit a complete evaluation of the true disease status of the joint and that the “tip of the iceberg” alone is observed.

REFERENCES: