Prevalence of naturally occurring cartilage defects in the ovine knee

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SUMMARY

Objective: To determine the prevalence, anatomical location and severity of cartilage defects in the stifle (knee) within a population of adult ewes (N = 65).

Materials and methods: Articular cartilage (AC) of the distal femur, proximal tibia and patella was assessed using Osteoarthritis Research Society International (OARSI) recommendations for macroscopic and microscopic scoring of ovine cartilage. Synovial fluid analysis and histology of the synovial membrane were performed. All limbs were examined by computed tomography.

Results: Twenty-eight sheep (n = 28; 43%) presented at least one score 2 or score 3 lesion. Twenty-two (n = 22; 34%) sheep were macroscopically normal. Most frequent localizations of lesions were: axial aspect of the central third of the medial tibial condyle (32.7% of the lesions), middle third of the medial femoral condyle (29.4%), middle third of the articular surface of the patella (9.8%), and axial aspect of the central third of the lateral tibial condyle (9.8%). Grade of macroscopic lesions was significantly (H (3) = 29.31, P < 0.000) affected by age. Macroscopic score correlated well with histological changes that can be found in osteoarthritis (OA) (r = 0.83; P < 0.000). Neither clinical signs of OA, nor cytological and histological signs of inflammation were identified, while imaging abnormalities were very rare.

Conclusions: Our data seem to indicate that naturally occurring OA exists in ageing sheep, at least subclinically. It might be useful to take into account prevalent cartilage defects at baseline in studies using ovine models.

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Introduction

Articular cartilage (AC) defects of the knee are common in humans and are encountered in approximately 60% of knee arthroscopies1. The extent of pathology ranges from small focal defects to widespread damage to the articular surface. In animals, there is limited information about the prevalence and impact of AC defects. Palmar osteochondral disease (POD)2 and AC defects in the metacarpophalangeal joint3 are prevalent in Thoroughbred racehorses. In sheep, microscopic lesions of osteochondrosis were reportedly to be frequent in fast-growing lambs although few defects progressed to gross lesions4. Spontaneous non-infectious osteoarthritis (OA) has not been reported in this species. Proteoglycan loss, cartilage matrix atrophy and erosion of the cartilage consistent with OA change occur spontaneously in the unprotected region of the medial tibial plateau in goats as young as 2 years of age5. As ovine models of knee OA are an essential modality for exploring the pathophysiology and therapy of the disease6–15, information about the prevalence of AC abnormalities in this joint would be useful.

The objective of this study was to determine the prevalence, anatomical location and severity of naturally occurring cartilage defects in the knee within a population of adult ewes enrolled for research.

Material and methods

Animals

Hind limbs (n = 130) from 65 crossed Texel ewes (N = 65), euthanatized for reasons other than hind limb lameness, were
disarticulated at the level of the coxo-femoral joint and collected within 6 h of euthanasia. Sheep ranged from 6 months to 11 years old (mean 5.9; standard deviation [SD] 3.1) and weighed between 35 and 72 kg (mean 57.5; SD 9.0). The animals came from the Ovine Research Centre of the University of Namur. The experimental protocol (KI 10/148) was approved by the local ethical committee for animal welfare. Sheep included in this study were euthanized from May 2011 to February 2013. Each limb was identified by a number.

Outcome measures

Computed tomography

Immediately after euthanasia, all limbs (n = 130) were examined with an Emotion 6 (Siemens). Acquisition protocol was: 130 KV, 80 mAs, pitch 0.4, collimation 0.6 mm and rotation time of the tube 0.6 s. Images of 0.6 mm were reconstructed with an increment of 0.3 mm. From this isotropic volume of images, coronal, sagittal and transversal reformations were obtained (1 mm thickness and 1 mm space between slices). Field of view extended from the base of the patella to 6 cm below the tibial plateau. Images were reconstructed on a 512–512 matrix, and in-plane resolution was 0.2 mm. Images were then transferred on a medical digital imaging system (PACS, TELEMIS). All reconstructed images were prospectively stored on CD roms. Later, the knees were examined by a specialist in medical imaging, who was not aware of the identity of the limbs, to identify osteophytosis, subchondral bone (SB) sclerosis and SB irregularities. Those features were evaluated as present or absent.

Synovial fluid (SF) analysis

The knee region was clipped and cleaned. Synoviocentesis was performed on both limbs of the first 50 (N = 50) sheep of this series by a paraligamentous approach using a 20-gauge needle. It was not performed on the last 15 individuals (N = 15) because of financial costs that would not have been justified considering the absence of findings in the initial 50 sheep. In those animals, when SF was obtained, volume was measured. Samples were placed in tubes containing ethylenediaminetetraacetic acid (EDTA) for routine SF analysis. Total blood cell count and enumeration were performed by flow cytometric method (ADVIA 120® – Siemens). Total protein concentration was measured by spectrophotometry (DPP® – Roche). Cytology results were checked by microscopic examination and manual counting using a hematocytometric cell.

Histology of the synovial membrane

Also, in the first 50 sheep of this series (N = 50), samples of synovial membrane were harvested in each knee (n = 100) from the suprapatellar fold. Specimens were fixed in 10% (v/v) neutral buffered formalin and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (H&E). Several parameters were observed for their presence: intima hyperplasia, lymphocytic or plasmocytic cellular infiltrate, sub-intimal fibrosis and vascularity.

Gross observation

Soft tissue was removed, and the knee joint was carefully disarticulated. The cartilage was kept moist by covering the joint surface with gauze sponges soaked in lactated Ringer’s solution. The distal articular surface of the femur, proximal articular surface of the tibia and articular surface of the patella were examined by gross observation by two investigators. Joint surfaces were digitally photographed with standardized lighting conditions for records. Both investigators, working in consensus, identified and scored the abnormalities of the AC. Macroscopic scoring was done in a blinded manner, such that the age and the sheep identity (i.e., leg pairing) were unknown to the scorers. Osteoarthritis Research Society International (OARSI) recommendations for macroscopic scoring of cartilage in sheep were used: score 0 for intact cartilage surface; score 1 for surface roughening; score 2 for deeper defects (fibrillation, fissures) not involving the SB; score 3 for erosions down to SB (less than 5 mm diameter); score 4 for large erosions down to SB (more than 5 mm diameter). Scoring of articular surfaces was performed in 26 anatomic areas of each knee (Fig. 1). In a second phase, two other investigators reviewed the specimens and compared their scoring to the first list of lesions. Discrepancies were discussed between all investigators till a consensus was reached. For macroscopic observation, the most severe lesion observed in one region was used to score the articular surface of that region. Grading of cartilage defects within each knee (“knee grade”) was obtained by the addition of the scores of all regions in that knee. A “sheep grade” was also calculated by addition of the grades from the paired legs.

Histopathology of cartilage

Three to four millimetre thick osteochondral slabs were obtained for every lesion, centred on the articular surface defect, and were processed for histology. Samples from sites with no macroscopic abnormality (macroscopic scoring of 0) were also obtained for control. These control samples were randomly selected among the articular surfaces which were diagnosed healthy at macroscopic observation, and in a number similar to the total number of the lesions identified in the 65 sheep (N = 65). Samples were fixed in 10% (v/v) neutral buffered formalin for 48 h. Following fixation, samples were transferred to 70% (v/v) ethanol for storage or further processing. They were decalcified in DC3 (non-ionic surfactants, chlorhydric acid, EDTA) for 2 days and embedded in paraffin. Four-micron sections were cut. Sections were de-paraffinised with xylene and graded ethanol, and then stained with toluidine blue.

Histological analysis was carried out independently by two investigators. Discrepancies were discussed till a consensus was reached. Sheep, age, limb identity and macroscopic score were unknown by the histological scorers. Scoring was performed using the OARSI recommendations for histological evaluation of AC in sheep. Histological abnormalities seen in osteoarthritic cartilage include structural defects, chondrocyte cloning and death, loss of proteoglycan, calcified cartilage layer (CL), thickening and tidemark duplication, penetration of blood vessels through SB plate and tidemark, sclerosis of the SB.

The objectives of histological analysis were (1) to assess cartilage structure and to evaluate the accuracy of macroscopic observation (for example, to confirm whether a score 2 lesion at gross observation correlated with a partial thickness defect [fibrillation, fissures] not involving the SB at microscopy), (2) to identify any evidence of other abnormalities (other than structural changes) present in OA, and (3) to evaluate whether histological features of OA cartilage pathology were present in normal (non-lesion) cartilage.

Statistical analysis

Data for analysis included 65 (N = 65) “sheep grades”, 130 (n = 130) “limb grades” and CT observations, 100 (n = 100) SF and synovium analyses, 153 (n = 153) macroscopic scores of lesions and 268 (n = 268) histological scores (cases and controls). Kolmogorov–Smirnov and Shapiro–Wilk tests were used to examine the normality of data. Due to the non-normality of data, non-parametric tests were used. Correlations between variables were assessed by using the Spearman correlation coefficient. The Wilcoxon signed-rank test and the Friedman test were used to
compare observations that were not independent ("limb grades", anatomical locations). The Kruskal–Wallis test was used to compare the mean ranks of "sheep grades" in different categories of age. Data were collected in Microsoft Excel and were analysed using IBM® SPSS® Statistics, version 15 for Windows. A P-value less than 0.05 was considered to indicate a statistically significant difference.

**Results**

**Macroscopic findings**

One hundred and fifty three cartilage defects were detected over 43 (n = 43) of the 65 (N = 65) sheep in 13 anatomical locations (Table I). Twenty two (n = 22) sheep were macroscopically normal. They were 70 score 1 (45.8%), 76 score 2 (49.7%), and seven score 3 (4.6%) lesions. Twenty eight sheep (n = 28; 43%) presented at least one score 2 or score 3 lesion. Most frequent localizations of lesions were: axial aspect of the central third of the medial tibial condyle (TCM2Ax; 32.7% of the lesions), middle third of the medial femoral condyle (FCM2; 29.4%), middle third of the articular surface of the patella (P2; 9.8%), and axial aspect of the central third of the lateral tibial condyle (TCL2Ax; 9.8%). Mean macroscopic scores were 1.50 (SD 0.08) for TCM2Ax, 1.58 (SD 0.08) for FCM2, 1.93 (SD 0.15) for P2, and 1.53 (SD 0.13) for TCL2Ax. Examples of gross anatomic lesions are shown in Fig. 2.

Age, weight and "sheep grades" are reported in Table II. Mean "sheep grade" was 3.83 (SD 4.99; range 0–23). There was no significant difference in "limb grades" between left and right limbs. There was no correlation between weight and "sheep grade", while age and sheep grades were well correlated (r 0.675; P 0.000). "Sheep grade" was significantly (H (3) = 29.31, P 0.000) affected by age (1–3 year old, 4–6, 7–8, 9–11), with mean ranks of 16.21, 28.09, 39.54 and 50.79 respectively. Table II shows that no lesion was found in the eleven (N = 11; 100%) sheep less than three year old, while at least one score 2 or 3 lesion was found in 15 (N = 15) of the 17 (N = 17), i.e., 88%, sheep aged between nine and 11. Computed tomography did not identify any signs of SB sclerosis or osteolysis. Osteophytes were found in only two animals (N = 2). One sheep was 11 year old and had a "sheep grade" of 23, while the other was seven and had a "sheep grade" of 18. Osteophytes were bilateral (left and right limb), small and present on the medial aspect of the tibial plateau. CT identified SB sclerosis only in this 11-year-old sheep.

**Table I**

<table>
<thead>
<tr>
<th>Localization</th>
<th>n</th>
<th>% of n total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCM2Ax</td>
<td>50</td>
<td>32.7%</td>
</tr>
<tr>
<td>FCM2</td>
<td>45</td>
<td>29.4%</td>
</tr>
<tr>
<td>P2</td>
<td>15</td>
<td>9.8%</td>
</tr>
<tr>
<td>TCL2Ax</td>
<td>15</td>
<td>9.8%</td>
</tr>
<tr>
<td>G2</td>
<td>8</td>
<td>5.2%</td>
</tr>
<tr>
<td>FCM3</td>
<td>5</td>
<td>3.3%</td>
</tr>
<tr>
<td>FCL2</td>
<td>5</td>
<td>3.3%</td>
</tr>
<tr>
<td>G1</td>
<td>3</td>
<td>2.0%</td>
</tr>
<tr>
<td>TL2</td>
<td>2</td>
<td>1.4%</td>
</tr>
<tr>
<td>FCL1</td>
<td>2</td>
<td>1.3%</td>
</tr>
<tr>
<td>TCM2Ab</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>P1</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>P3</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

**Table II**

| Distribution of localizations of lesions (n = 153) in this series of 65 sheep (N = 65). Proximal, middle and distal thirds of the articular surface of the patella (P1, P2, P3); proximal and middle thirds of the groove of the trochlea of the femur (G1, G2); middle and distal third of the lateral ridge of the trochlea of the femur (TL2); middle third of the articular surface of the medial femoral condyle (FCM2, FCM3); cranial and middle thirds of the articular surface of the lateral femoral condyle (FCL1, FCL2); middle third of the articular surface of the medial tibial condyle, divided into axial and abaxial aspects (TCM2Ax, TCM2Ab); middle third of the axial aspect of the articular surface of the lateral tibial condyle (TCL2Ax). |
Microscopic findings

SF analysis and synovium histopathology

No synovial effusion was identified in all limbs. In 93 (n = 93) of the 100 knees (n = 100) where arthrocentesis was performed, no SF could be obtained. The volume of SF collected from the seven (n = 7) remaining knees (from five different sheep) varied between 0.5 ml and 1.2 ml (0.93 ± 0.26 SD). All samples were transparent, clear to pale yellow, and highly viscous, except one which was slightly discoloured by blood. Total protein concentration was within normal limits (2.5 – 5.0 g/100 ml) and varied from 1.69 to 3.41 g/100 ml (mean 2.45 ± 0.73 SD). Red blood cell (RBC) count was within normal limits (<10,000/mm³) except in the contaminated sample (50,000/mm³). White blood cell (WBC) count was within normal limits (<5,000/mm³) and varied from 30 to 300 (116.67 mean ± 110.03 SD). Histological analysis of the synovium that was sampled in the 100 limbs (n = 100) did not identify any characteristics of inflammation.

Cartilage histopathology

A total of 153 (n = 153) anatomic sections were obtained at the level of the 153 (n = 153) lesions identified. Random selection of control sites produced respectively 130 (n = 131) one slices. On a total of 284 (n = 284) histological slices, 16 (n = 16) presented artefacts or inadequate colouration and were not used for analysis. Correlation between histological structural score and macroscopical score was very good (Spearman coefficient, 0.83; P < 0.000).

Table III summarizes the histological findings (structure, chondrocyte density, cell cloning, inter-territorial blue, tidemark and extension of lesion) found in every level of macroscopic score.

Table III

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>0.68 (0.12)</td>
<td>3.43 (0.32)</td>
<td>6.86 (0.23)</td>
<td>9.7 (0.18)</td>
</tr>
<tr>
<td>Chondrocyte density</td>
<td>0.36 (0.07)</td>
<td>1.15 (0.14)</td>
<td>2.78 (0.18)</td>
<td>3.86 (0.14)</td>
</tr>
<tr>
<td>Cell cloning</td>
<td>0.50 (0.08)</td>
<td>1.04 (0.15)</td>
<td>2.93 (0.18)</td>
<td>4.00 (0.01)</td>
</tr>
<tr>
<td>ITB</td>
<td>0.31 (0.06)</td>
<td>1.12 (0.16)</td>
<td>2.56 (0.16)</td>
<td>3.57 (0.30)</td>
</tr>
<tr>
<td>Tidemark</td>
<td>0.53 (0.08)</td>
<td>1.18 (0.14)</td>
<td>1.64 (0.13)</td>
<td>2.14 (0.34)</td>
</tr>
<tr>
<td>Extension</td>
<td>0.26 (0.10)</td>
<td>1.34 (0.20)</td>
<td>2.69 (0.17)</td>
<td>3.86 (0.70)</td>
</tr>
<tr>
<td>Total</td>
<td>2.65 (0.38)</td>
<td>9.25 (0.95)</td>
<td>19.4 (0.86)</td>
<td>27.14 (0.80)</td>
</tr>
</tbody>
</table>
Total histological score was significantly different between localizations of interest (TCM2Ax, TCI2Ax, FCMI2, P2) ($\chi^2 = 53.87$, df 1, P<0.000) with mean scores of 14.33 (SD 1.23), 11.61 (SD 2.00), 9.80 (SD 1.28), 9.63 (1.78) respectively.

Discussion

The population of sheep in the “Centre de Recherche du Mouton” includes 450 ewes producing about 1,000 lambs a year. Besides research in reproduction, retired animals are used for orthopaedic research and teaching. Retirement is usually due to non-orthopaedic diseases (such as mastitis, metritis, loss of fertility) and occurs between five and 11 years. In this study, younger animals were also used so that a larger range of ages was considered to assess the prevalence of cartilage defects, and the effect of age. This study demonstrated that score 2 and 3 cartilage defects were prevalent in 43% of animals in this population of sheep, and they were correlated with histological changes that can be found in OA. However, no clinical signs of OA were present, such as joint effusion and lameness. SF and synovium analysis did not reveal any sign of inflammation. In addition, changes like osteophytes and SB sclerosis were nearly never identified by CT.

The clinical significance of those AC defects remains to investigate. In man, it may seem intuitive that, due to the limited healing ability of AC, the knee defects can only worsen over time; however their natural progress and their clinical significance has not yet been clearly established. The severity of symptoms attributable to chondral defects does not correlate with the size of the defect: small defects can be as disabling as end-stage arthritis. Recent data also suggest that prevalent focal AC defects are a risk factor for future progression of OA. In Thoroughbred racehorses also, the significance of prevalent cartilage defects in the metacarpophalangeal joint has yet to be clarified.

Our cross-sectional study does not indicate whether the lesions may progress in severity, or whether they can resolve. Longitudinal studies investigating the progress of those lesions will be necessary to understand their clinical significance. The absence of clinical signs (synovial effusion, lameness) and histological abnormalities of the synovium suggest that those defects were not associated with an active inflammatory process of the joints at the time they were assessed.

In humans, one common factor underlying osteochondral pathology is an increase in biomechanical stress leading to cartilage deterioration. From an epidemiologic perspective, several biomechanical risk factors have been demonstrated: overloading of joints due to obesity, angular deformity, joint dysplasia, meniscal tears, or occupational activities. In horses, the cartilage degeneration index increases from lateral to medial and from central to dorsal in the metacarpophalangeal joint: this specific distribution pattern strongly supports the important role for biomechanical loading in the distribution of pathology across the joint. In sheep, almost constant distribution of the joint force (about 1.7 body weight) is loaded on the medial tibial plateau during the maximum loaded phase of ovine gait, while only about 0.6 BW is carried laterally. A significantly larger contact area of 107.7 ± 28.7 mm² on the medial plateau than on the lateral plateau (60.8 ± 56.3 mm²) was reported. Furthermore, cartilage thickness is greater on the medial plateau by a ratio of 2.19. In the current study, the defects were more frequently identified in FCMI and TCM2Ax. Those sites correspond to the medial femoro-tibial compartment, suggesting that cartilage defects may occur where higher loads are expected. This reinforces the hypothetical link between cartilage defects and mechanical loadings.

Weight and obesity have been associated with a higher prevalence of knee OA in humans. In this study, no correlation was observed between weight and “sheep grade”. Though the number of animals was significant, they were not pure Texel and it is possible that this induced variations of conformations (size and weights) limiting objective evaluation of the impact of weight. Body mass index would be a more useful variable.

Topographical differences between the left and right knee have been reported in the sheep. The authors concluded that the medial tibial slabs of the right knee had thinner AC than the left medial tibial slabs. This difference is difficult to explain. In our study, no significant differences between right and left limbs were identified.

Exercise and type of exercise also play a role in the development of cartilage lesions in sheep. Lambs that were circled on an 8.5 m circle showed greater macroscopic evidence of lesion development compared to straight-line exercised and non-exercised control sheep. Palmer showed that concerted efforts by producers to change conformation, reduce exercise, and maximize growth rates should eventually precipitate clinical case of osteochondrosis. In our study, all sheep had a normal life in pasture and the same natural level of exercise.

The protective effect of sex hormones on cartilage has been demonstrated in sheep. In our study, only females were investigated. The reason is that usually only males used for breeding are kept; others are sold at young age on the food market. Interestingly, though not included in this series of animals, one 8-year-old male was dissected (for teaching purposes) and had score 3 lesions in the knee. It would be interesting to test the association between sex and prevalence of cartilage defects in future studies. However, higher level of activity in males associated with breeding (copulation) might constitute a confounder.

Macroscopic score correlated well with histological score of OA. It was significantly associated with age in this population. For example, while no lesion was found in sheep less than three year old, at least one score 2 or 3 lesion was found in 88% of sheep aged between 9 and 11. Our data seem to indicate that OA exist in ageing sheep. However, the identified morphologic changes were associated neither to clinical signs of OA, nor to cytological and histological signs of inflammation. In research, there is a perceived advantage in using naturally occurring models of OA in that they are more like human OA with slower onset and progression. OA is a disease of ageing, but age alone does not cause OA; rather, the vulnerabilities of the joint that occur as part of ageing make the joint susceptible to disease. Thus, to obtain the most meaningful insights into human OA, it might be interesting to consider the use of old animals (after 8 year old for example) as well as to evaluate whether exercise could exacerbate the development of subclinical and clinical OA in adults between 4 and 8 year old. As macroscopic and histological scorings of AC abnormalities are essential outcome measures in research studies using ovine induced models, our results suggest that, in a population of crossed-bred Texels used for research, it might be useful to take into account prevalent cartilage defects at baseline.

Author contributions

The initiation, conception and planning of this study was by J.-M.V., J.-F.N. and P.G. Its execution was by J.-M.V., J.-F.N, P.G., F.H., and P.C. Critical revision of the article for important intellectual content was by P.C.

Competing interests

None of the authors of this paper has a financial or personal relationship with people or organizations that could inappropriately influence or bias the content of the paper.
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References