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Research article

ASSOCIATION OF CARTILAGE, SYNOVIAL FLUID AND MEMBRANE PATHOLOGICAL FINDINGS IN SERBIAN MOUNTAIN HORSES WITHOUT SIGNS OF LAMENESS

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Osteoarthritis (OA) in horses often affects the metacarpal/metatarsophalangeal (MCP/MTP) joints and its detection in the early stages is crucial for effective management. It was hypothesized that the extent of cartilage damage positively correlates with synovial membrane (SM) and synovial fluid (SF) pathological findings in the MCP/MTP joints of Serbian mountain horses that transported heavy loads but did not show signs of lameness. The study was conducted on 32 MCP/MTP joints of eight horses between seven to 12 years of age. Horse limbs were transferred from the abattoir to the necropsy room and SF was sampled. Its appearance, total nucleated cell count (TNCC), mononuclear cell count (MNC), total proteins (TP), and haptoglobin (Hp) were determined. Samples of SM were collected from the dorsal palmar/plantar pouch for histology. A macroscopic examination of gross condylar pathology of the third metacarpal/metatarsal bone was performed with Indian Ink staining. Scoring was done based on Osteoarthritis Research Society International recommendations. SF was clear, pale yellow, and mostly fairly viscous. Half of the samples had TNCC above, and all had TP within the reference range. Hp values were below the reference range and were omitted from further analyses. TNCC correlated with MNC ($\varrho s=0.81$, $P<0.001$), microscopic ($\varrho s=0.62$, $P=0.003$) and macroscopic scores ($\varrho s=0.47$, $P=0.008$). In addition, MNC correlated with macroscopic scores ($\varrho s=0.40$, $P=0.03$). All pathological findings were mild and their correlation indicated that these processes are interrelated and that could be ascribed to early OA.

Keywords: Serbian mountain horses, early osteoarthritis, synovial fluid, synovial membrane

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INTRODUCTION

Osteoarthritis (OA) is a common joint disease in horses, which negatively impacts their working capacity and overall well-being [1]. OA affects all joint structures and leads to the loss of articular cartilage, joint capsule thickening, inflammation of the synovial membrane, subchondral bone sclerosis, and osteophyte formation, which ultimately results in loss of joint function [1,2]. As MCP and MTP joints carry the horse's weight they are prone to develop OA [3]. In jumping and thoroughbred horses, OA of the MCP/MTP joints can cause significant lameness, making training and competition impossible and therapy ineffective [4-6]. Also, it is known that OA treatment is most effective during its early stages [7,8].

In early OA no clinical signs are visible, but specific biomarkers in the body fluids and/or changes in non-invasive imaging diagnostics are present [9]. These early stages of OA are not well understood and are often overshadowed by the more advanced and symptomatic phases. This is partly because laboratory animal models of OA do not exhibit the early stages of the disease due to artificially induced injury [10]. The only way to diagnose naturally developed early OA in large animals is through techniques such as magnetic resonance imagining (MRI) or arthroscopy and by analyzing biomarkers present in the synovial fluid. The ideal biomarker changes should occur before any morphological changes, enabling early diagnosis [11]. Hp is one of the biomarkers tested several times in experimentally induced OA in horses [12,13]. However, its clinical utility in diagnosing early OA has not yet been determined.

Serbian mountain horses possess a solid build, with high resistance and endurance levels [14]. They are commonly used as hard-pack animals to transport wood on difficult terrains. The load they carry is often as heavy as one-fifth of their weight, which can lead to repeated muscle and joint injuries. It is established that excessive mechanical loads can cause one of the six clinical phenotypes of OA [15]. Our study the aim was to determine the extent of cartilage surface damage in MCP/MTP joints of working horses without lameness. Additionally, we seek to investigate if these changes correspond with histological evidence of synovial membrane alterations and synovial fluid parameters that can indicate early onset of OA.

MATERIAL AND METHODS

Study Population and Clinical Examination

In Serbia, the demand for horse meat is increasing. Due to this, some working horses are being sent to abattoirs. The lower part of the limbs below the carpal and tarsal joints are not used for human consumption and are considered a by-product convenient for research purposes. The limbs for this study were collected at an abattoir equipped with a stable for the animals' rest after transportation.

Serbian Mountain working horses that arrived at the abattoir between March and June 2019 were enrolled for the study. Their veterinary health certificates served to obtain their provenance, age, and vaccination status. It is known that these working horses in the mountain areas of Serbia begin carrying loads when they are two – to three-year-olds. They had a period of six to 12 months of adaptation, during which they only walked alongside the adult working horses. The working months were from May to October. During the day, the horses free of burden climb to hilly woods and in the afternoon go down the hills with their load. Before going to the abattoir, their nutrition was optimized for several months to achieve a good body condition score (BSC). The BSC estimation was done according the scale from 1 (poor) to 9 (extremely fat). The horses' body weight was measured at a livestock scale of the abattoir.

The horses also had to be free of any orthopedic problems or obvious lameness while resting after transport. The orthopedic examination was performed according to the guidelines of the American Association of Equine Practitioners [16]. The movement inspection was performed on firm hard ground on walk and trot in a straight line. No flexion test was performed. If any abnormalities were noted, the horses were excluded from the study. In total, 32 limbs were collected from eight horses.

Sample collection and macroscopic cartilage inspection

Immediately after slaughter, limbs were removed from the carpus/tarsus region and within one hour transported on the cold chain to the necropsy room at FVMUB. The further sampling procedure was as follows:

1. Synovial fluid from MCP/MTP joints was collected under aseptic conditions by a 21G needle placed between the disto-palmar and disto-plantar metacarpal/metatarsal condyles and the dorsal part of the lateral proximal sesamoid bone [17]. A minimum of 3 ml of synovial fluid was retreated and transferred into sterile Eppendorf tubes without anticoagulant. Routine synovial fluid examination was carried out immediately and all the surplus of material was stored at – 80° C for a maximum of 30 days until further biochemical tests. One sample of synovial fluid was lost in the process of collection.
2. After synovial fluid sampling, the joints were dissected. Specimens of the synovial membrane, 5×5 mm in size, were collected from the dorsal palmar/plantar pouch of the fetlock joint capsule that extends proximally between the third metacarpal/metatarsal bone (MCIII/MTIII) and suspensory ligament, and fixed in 10% buffered formalin.
3. Further on, cartilage of the distal ends of the MCIII/MTIII bones was inspected using a blue ink staining procedure [18]. Photographs with a digital camera (Olympus C-7070 VIDE ZOOM) were taken before and after staining. Image J software (available online: https://imagej.net/ij/download.html) was used to measure the area of the mentioned lesions. The size of the lesions was expressed as a percentage of the total joint surface. Macroscopic evaluation of the wear lines, erosions, and palmar arthrosis was performed according to the recommendations

of the Osteoarthritis Research Society International (OARSI) using a scale ranging from 0 to 3. A maximum score for cartilage surface lesions of 9 could be obtained (Table 1).

Table 1. The macroscopic grading system of lesions on the cartilage surface [18].

Overall macroscopic change		
Lesions	**Grade**	**Description**
Wear lines	0	None
	1	1 or 2 partial–thickness wear lines/joint surface
	2	3-5 partial–thickness or 1–2 full–thickness wear lines/joint surface
	3	>5 partial–thickness or >2 full–thickness wear lines/joint surface
Erosions	0	None
	1	Partial-thickness erosion, <5 mm in diameter
	2	Partial-thickness erosion, >5 mm in diameter
	3	Full-thickness erosion
Palmar arthrosis (osteochondral lesions distal palmar aspect of metacarpus)	0	None
	1	Partial–thickness erosion, <5 mm in diameter
	2	Partial–thickness erosion, purple discoloration, >5 mm in diameter
	3	Full–thickness erosion, purple discoloration, >5 mm in diameter

Synovial fluid analysis

The color and transparency of synovial fluid were noted upon taking the sample. The viscosity was evaluated by qualitative estimation of granularity and withdrawing of cells on the thin-spread air-dried cytological slides made after well-mixing and staining with Romanowsky dye (Bio-Diff, Biognost, Croatia). The coarse granularity and withdrawing indicated good viscosity and was marked by 3+, borderline granularity without withdrawing indicated moderate/fair viscosity and was marked 2+, and homogeneous background with random cell distribution designated poor viscosity and was marked 1+ [19]. Estimation of the total nucleated cell count (TNCC) and differential mononuclear (MNC) and polymorphonuclear (PMN) count was done by visual assessment on smears, using an objective lens with 100 × magnification. TNCC was calculated using the formula:

Number of cells/μL = (the mean value of the number of cells in the visual field) x (microscope magnification)^2

Samples of synovial fluid with a pinch of hyaluronidase powder (Sigma H3884, Sigma-Aldrich, Saint Louis, Missouri, USA) were incubated for 20 minutes at 25 °C and centrifuged at 1400 × g for 15 minutes. TP concentration was measured from supernatant with standard biuret reaction (Elitech, Puteauque, France) on an automatic analyzer Technicon RA-KST (Bayer). The Hp concentration was determined by the spectrophotometry as previously described [20].

Histology analysis

Fixed synovial membrane samples were processed by standard histological procedures, embedded in paraffin, and cut into 5 μm sections. The sections were stained with hematoxylin/eosin staining (H/E) (Merck Millipore Darmstadt, Germany) and by the van Gieson (VG) staining method (Švob, 1974). Histologic assessment of the synovial membrane was performed according to the instructions of the OARSI [18] under the microscope (Olympus CKS31) equipped with a digital camera (UC50 Soft Imaging Solutions) and connected to appropriate software. The following parameters were evaluated: cellular infiltration, vascularization, intimal hyperplasia, subintimal edema, and subintimal fibrosis. Changes were graded with a maximum score of 20 as previously described [18]. The grading system for each parameter is presented in Table 2.

Table 2. The microscopic grading system of the synovial membrane [18].

Synovial membrane		
Outcome parameter	**Grade**	**Description**
Cellular infiltration (lymphocytes and plasma cells)	0	No mononuclear cells in the section
	1	Occasional small areas of mononuclear cells throughout the section
	2	Mild presence of mononuclear cells in 25% of the section
	3	Moderate presence of mononuclear cells in 25–50% of the section
	4	Marked presence of mononuclear cells in greater than 50% of the section
Vascularity	0	Normal
	1	Slight increase in vessels in focal locations throughout the section
	2	Mild increase in number and dilatation of vessels in focal locations throughout the section
	3	Moderate increase in number and dilatation of vessels in up to 50% of the section
	4	Marked increase in number and dilatation of vessels in greater than 50% of the section
Intimal hyperplasia	0	None
	1	Villi with 2–4 rows of intimal cells within the section
	2	Villi with 4–5 rows of intimal cells over 25–50% of the section
	3	Villi with 4–5 rows of intimal cells over 50% of the section
	4	Villi with 5 or greater rows of intimal cells over 50% of the section
Subintimal edema	0	No edema
	1	Slight edema detected within section
	2	Mild edema within 25% of the section
	3	Moderate edema within 25–50% of the section
	4	Marked edema in greater than 50% of the section
Subintimal fibrosis	0	Normal
	1	Slight increase in fibrosis within the section
	2	Mild increase in fibrosis in 25% of the section
	3	Moderate increase in fibrosis in 25–50% of the section
	4	Marked increase in fibrosis in greater than 50% of the section

Statistical analysis

Descriptive analysis was performed by calculating the median and reporting minimum and maximum values. Correlation analysis, was performed using the Spearman's rank

correlation coefficient. Significant difference was with P-values of < 0.05, P-values of < 0.01 and P-values of < 0.001. Data were analyzed using statistical software GraphPad Prism, version 7 (GraphPad, San Diego, USA).

RESULTS

In a period of four weeks ten horses arrived at the abattoir. Horses that did not have fever or any obvious physical changes after visual inspection, auscultation, palpation, and percussion, were included in the study. Movement inspection showed that eight horses did not have visible signs of lameness. Also, the limbs did not have skin lesions, wounds, or edema. Their body weight was in median=416.5 kg, (minimum=387.0 kg and maximum=452.0 kg) and they had moderate BCS (median=4.0, minimum=3.5 and maximum=4.5). Among them, four were males and four were females with a median age of eight and a range of seven to 12 years. They were engaged in lifting wood from four to nine years. Two horses were excluded. One was due to visible deformation of MCP/MCT joints and the other was younger than 3 years. None of the horses had a known vaccinal status.

Synovial fluid parameters

The gross appearance of synovial fluids was pale yellow and transparent (Table 3). The qualitative viscosity score in the majority of samples was fair and poor (Table 3).

Table 3. Synovial fluid analysis of MCP/MTP joints from fore and hind limbs of Serbian mountain horses (n=8).

Variables	Reference values	Total number of joints examined	Median value (main characteristics)	Min–Max	Beyond (↑) and below (↓) reference values (%)	95% CI
Color	Pale yellow	31	Pale yellow	N.A.	N.A.	N.A.
Transparency	Transparent	31	Transparent	N.A.	N.A.	N.A.
Viscosity (smear)	3	31	2	1-3	↓ 62.40	36.72–95.93
TNCC (/μL)	< 1000	31	1300	200–9400	↑54.84	36.03–72.68
MNC (/μL)	500–900	31	890	58–8460	↑64.29	44.07–81.36
PMN (/μL)	< 100	31	5.5	1.0–40.0	0	0
Total proteins (g/L)	< 20	31	9.7	5.4–19.9	0	0
Haptoglobin (g/L)	1.0–2.5	30	0.14	0.03–2.31	N.A.	N.A.

Abbreviations: TNCC–total nucleated cells count; **PMN**–polymorphonuclear leukocyte; **MNC**–mononuclear cells; Min–Max–minimum to maximum values **N.A.**–not applicable. TNCC, MNC, PMN, and total proteins reference values for are based on [19], and for haptoglobin on [13]. The coarse granularity and withdrawing indicated good viscosity and is marked by 3, borderline granularity without withdrawing indicated moderate/fair viscosity and is marked by 2, and homogeneous background with random cell distribution designated poor viscosity and is marked 1 [19].

The TNCC was slightly above the reference range at approximately half of synovial fluid samples. The higher number of TNCC was the consequence of a higher number of mononuclear cells, while the absolute number of polymorphonuclear leukocytes (PMN) and the concentration of TP were in the reference range. The median concentration of Hp was 0.14 g/L within a wide range of values (Table 3). No statistically significant differences were found by comparing investigated cytological and biochemical parameters between the MCP/MTP joints of the forelimbs and hindlimbs or between the MCP/MTP joints between the left and right limbs.

Macroscopic evaluation of joint cartilage

Surface cartilage lesions were stained dark blue with Indian Ink in contrast to the light blue surface of undamaged cartilage (Figure 1).

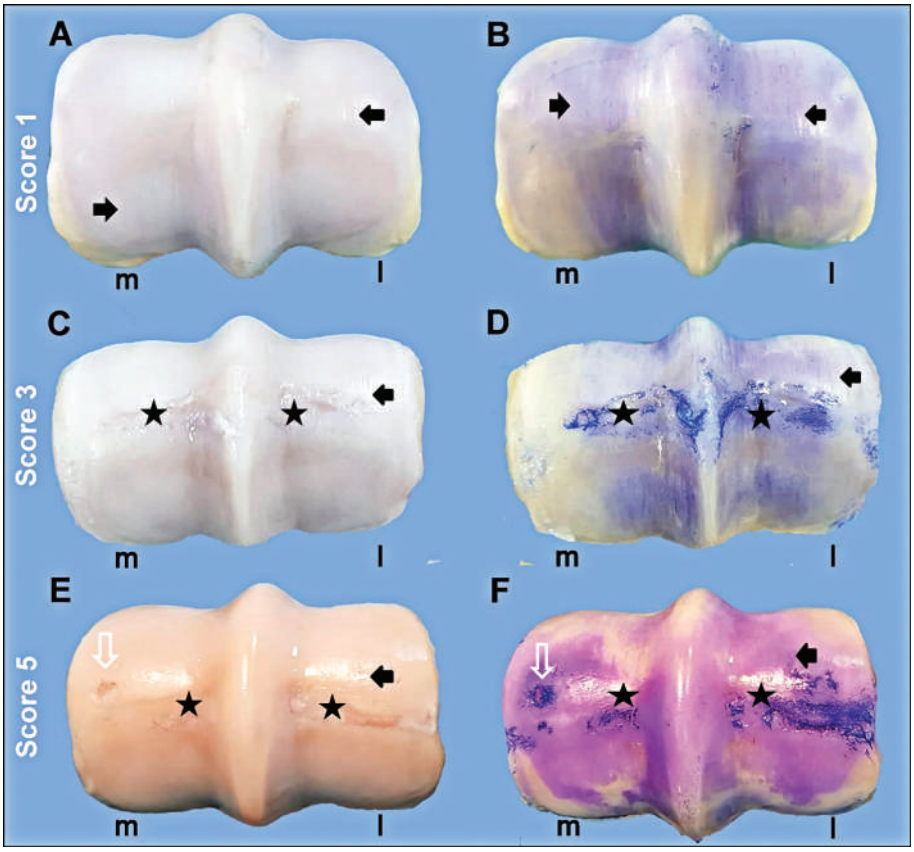

Figure 1. Macroscopic lesions on the third metacarpal bone before and after Indian ink staining: score 1 (**A, B**), score 3 (**C, D),** and score 5 **(E, F**). Images **(A, B)** show a single wear lines on the articular cartilage (score 1, black arrows). Images **(C, D)** show wear lines (score 1, black arrows) and palmar arthrosis (score 2, stars). Images **(E, F)** demonstrate the presence of wear lines (score 1, black arrows), erosion (score 1, white arrows), and palmar arthrosis (score 3, stars); **m**–medial condyle; **l**–lateral condyle.

Macroscopic lesions were present on all the cartilage surfaces of distal articulation of the MCIII/MTIII bones. About one-third of examined joints had a score of 3, while only one joint each had a score of 6, 7 and 8 (Table 4). It was observed that 81.3% (26 of 32 joints) lesions were wear lines (Figure 1 A, B, C, D, E, F), another 37.5% (12 of 32 joints) lesions were erosions (Figure 1 E and F) and 18.8% (6 of 32 joints) were palmar arthrosis (Figure 1 C, D, E, F). Significant differences were not found by comparing the macroscopic lesion scores between the MCP/MTP joints of forelimbs and hindlimbs or between the left and right limbs. No differences were observed in the damaged cartilage surface between the medial and lateral condyles (p=0.24) in the forelimbs and hindlimbs (p=0.18), and left and right limbs (p=0.21).

Table 4. Summary of macroscopic scores of MCP/MTP joint examination of Serbian mountain horses (n=8) based on joint gross pathology.

Macroscopic scores	n	n (%)	95% CI
1	2	6.25	0.77–20.81
2	7	21.88	9.28–39.97
3	9	28.13	13.75–46.75
4	7	21.88	9.28–39.97
5	4	12.50	3.51–28.99
6	1	3.12	0.08–16.22
7	1	3.12	0.08–16.22
8	1	3.12	0.08–16.22
9	–	–	–
Total	32	100	

Abbreviations: n–absolute number of MCP/MTP joints; **n (%)**–percentage of MCP/MTP joints; **95%CI**–confidence interval.

Histological evaluation of synovial membrane

The histologic examination was performed on 21 synovial membrane samples. The grading ranged from 1 to 4 with a possible total score of 20. In our study, maximum score was 9 (Table 5). It was observed that 67% (14 of 21 synovial membranes) had only minor changes (score 3). The number of samples with more severe changes gradually declined, with only one sample having a score of 9. Leukocyte infiltration (4.76%) and subintimal edema were rarely noticed (14.28%) (Figure 2. A, A1, A2). By evaluating the intimal hyperplasia (23.8%), it has been noticed that most of the examined forelimbs and hindlimbs have the mildest degree of changes, which were characterized by villi with 2 to 4 layers of synoviocytes (Figure 2. B, B1, B2). The majority of samples (85.71%) had focally visible abundant subintimal and deep vascularization dispersed throughout the section (Figure 2. C, C1, and C2). All samples except one (95.23%) had an accumulation of collagen fibers that did not have a recognizable pattern and differed between joints and animals (Figure 2. D, D1, and D2).

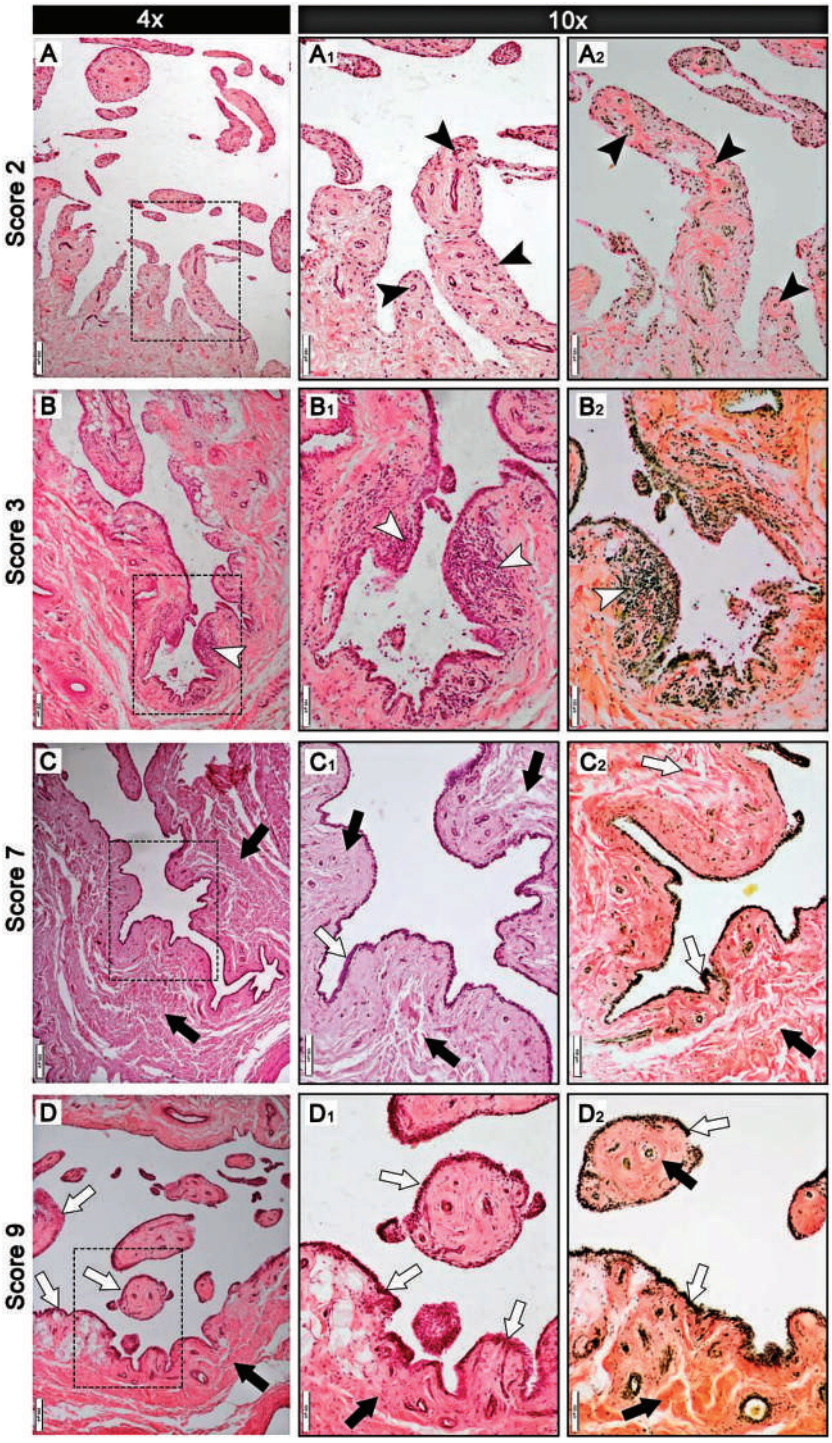
4x
10x
Score 2
Score 3
Score 7
Score 9
A
A1
A2
B
B1
B2
C
C1
C2
D
D1
D2

Figure 2. Representative photomicrographs of the synovial membrane section sampled from Serbian mountain horses' dorsal palmar/plantar pouch of the metacarpophalangeal and metatarsophalangeal joints. Photomicrographs showcase sections with different grades of histological changes: score 2 (**A**, **A_1**, **A_2**), score 3 (**B**, **B_1**, **B_2**), score 7 (**C**, **C_1**, **C_2**) and score 9 (**D**, **D_1**, **D_2**). The square with dashed lines represents the regions of the synovial membrane viewed with low power (10×) objective lens, which is shown in appropriate black-bordered pictures (**black arrowhead** – vascularity; **white arrowhead** – cellular infiltration; **black arrow** – subintimal fibrosis; **white arrow** – intimal hyperplasia). Tissue sections stained with hematoxylin/eosin viewed with a scanning (4×) objective lens (A, B, C, D) (bar: 200 μm) and low power (10×) objective lens (A_1, B_1, C_1, D_1) (bar: 100 μm) and sections stained with Van Gieson staining method viewed with low power (10×) objective lens (A_2, B_2, C_2, D_2) (bar: 100 μm).

Table 5. Summary of microscopic scores of MCP/MTP synovial membrane examination of Serbian mountain horses (n=8) based on histology analyses.

Microscopic scores	n	n (%)	95%CI
1	1	4.76	0.12–23.82
2	8	38.10	18.11–61.56
3	5	23.81	8.22–47.17
4	3	14.29	3.05–36.34
5	2	9.52	1.18–30.38
6	–	–	–
7	1	4.76	0.12–23.82
8	–	–	–
9	1	4.76	0.12–23.82
Total	21	100	

Abbreviation: n–absolute number of synovial membrane samples; **n (%)**–percentage of synovial membrane samples; **95%CI**–confidence interval.

Correlations between different measured parameters

A moderate positive correlation between TNCC and microscopic scores of synovial membrane, and macroscopic scores of cartilage lesions was found (Table 6). Also, macroscopic and microscopic scores correlated well (Table 6). TNCC was in high correlation with MNC and moderate with PMN (Table 6). Total proteins did not correlate with any parameters. Viscosity and Hp were not correlated with other parameters. None of the investigated parameters correlated with the age and weight of the horses.

Table 6. Spearman's correlation analysis between the investigated variables.

Variables	ϱs	P–value
TNCC21 and microscopic score21	0.62	0.003
TNCC31and macroscopic score31	0.47	0.008
Microscopic21 and macroscopic score21	0.55	0.009
TNCC31 and MNC31	0.81	<0.001
TNCC31 and PMN31	0.53	0.030
MNC31 and PMN31	0.49	0.040
MNC31 and macroscopic score31	0.40	0.030

Abbreviations: TNCC–total nucleated cell count; **PMN**–polymorphonuclear leukocyte; **MNC–** mononuclear leukocyte; ϱ_s–Spearman's rho rank correlation coefficient.The number in italics superscript designates the number of analyzed samples. Total proteins did not have any correlation with other parameters.
** Correlation is significant at the 0.01 level (2–tailed).
* Correlation is significant at the 0.05 level (2–tailed)

DISCUSSION

The study found that Serbian Mountain working horses without lameness have slight cartilage changes in their MCP/MTP joints. These changes are linked to the alterations in the microanatomy of the synovial membrane and an increase in synovial fluid TNCC and MNC. The findings suggest that the composition of synovial fluid mirrors other joint tissue pathologies [21].

The gross appearance of synovial fluid was in line with the physiological one. However, the viscosity of SF was not scored as "good" indicating a misbalance in the structure of hyaluronic acid and proteoglycans [19]. In addition, more than half of the joints had slightly higher TNCC than the upper reference limit suggested [19]. This mildly to moderately high TNCC was the result of a mononuclear cell increase. These cells belong to the monocytes/macrophages lineage or desquamated synoviocytes. A steady state number of PMNs and concentration of proteins together with normal gross fluid appearance confirmed our clinical observations and excluded the existence of acute inflammatory insult. The observed type of change is consistent with degenerative or early OA [19,22].

The concentrations of Hp were found to vary widely, spanning three orders of magnitude. However, the median value was in line with values obtained for Shetland ponies using the radial immunodiffusion method [12]. Although Hp appears to be a

promising biomarker that may be synthesized within a joint, there is currently a lack of standardized methods and reference ranges. We used a simple colorimetric method based on peroxidase activity [20] to determine Hp concentration, which has already been tested for horse synovial fluid in steady state and experimental arthritis [13]. However, this method may not be suitable for very low Hp concentrations due to its low specificity [23]. Therefore, we are unable to confidently discuss the Hp results of this study but we consider reasonable to suggest the use of immunoassays to test synovial fluid Hp concentration for early OA detection.

Significant cartilage damage was not detected only in two out of 32 joints. The most common finding was surface wear, which is considered the first stage of degenerative cartilage damage [18]. The lesions in working Serbian Mountain horses were evenly distributed over MCIII/MTIII and there was no difference in the distribution of these lesions between the forelimbs and hindlimbs, or the lateral and medial condyles. The Serbian Mountain horses included in this study were primarily used as pack animals, carrying loads of around 100 to 150 kg on their backs and they developed anatomically uniform lesions. However, based on present data, it is not possible to explain this uniformity. In contrast, draft horses and trotters that pull loads behind them tend to experience more degenerative cartilage changes on their hindlimbs [24]. Thoroughbreds, on the other hand, experience cyclic load and overload during racing and training which often results in forelimb cartilage injury at the distal ends of the MCIIII bones [25]. Moreover, in the same purebred horses, changes are more intense on the medial condyles of the forelimbs and lateral condyles of the hindlimbs [26].

Accumulation of collagen fibers in the synovial membrane indicates subintimal fibrosis. Besides chronic inflammation and autoimmune diseases, mechanical stress can promote fibroblast response and induce fibrotic changes in the synovial membrane in the initial stages of OA [27]. In addition, abundant vascularization and intimal hyperplasia with multiple layers of synoviocytes are present in the majority of the examined samples. In a steady state, only two layers of synoviocytes form the synovial membrane intima [28]. Thus, multiple layers of synoviocytes may represent reactive proliferation that also occurs in early OA [29]. Although the number of blood vessels might represent the tissue reaction, no PMN infiltration suggests the absence of an acute inflammatory process. A positive correlation between the macroscopically visible cartilage lesions and synovial membrane changes detected on histology shows the continuum of changes in the joints that most probably characterize an early OA process.

In our study, the observed pathology findings did not correlate with age, but it is also possible that the age of the horse did not align with the length of time spent working in extreme conditions. Also, it could be hypothesized that not all horses had the same workload over time they spent working.

The hallmark symptom of OA is pain. However, pain is a subjective sensation and is not always correlated with the stadium of OA. Thus, the absence of lameness in this

study excludes intensive pain, although low-intensity pain might be present and was not excluded with the flexion test. Therefore, based on clinical and laboratory findings it was evident that horses did not have a severe form of OA. Described pathological findings are consistent with early OA.

The limitations of the study are several:

The history of horse workload and work conditions is not complete. Extreme stress on the joints on steep slopes, unfavorable field conditions like mud or rocky ground, possible unfavorable frequency of rest breaks, and inadequate nutrition could all affect the development of joint damage. However, it was not possible to obtain these data retrospectively, at the abattoir. Thus, the real physical and mechanical problem behind the pathological findings cannot be unraveled.

The flexion test, an additional lameness exam that accentuates pain, was not performed due to the short time spent with horses in the abattoir. Due to this limitation, we were not able to have a complete clinical examination that could relate our laboratory findings with clinical ones.

CONCLUSION

The standard analysis of synovial fluid from the MCP/MTP joints of Serbian Mountain working horses without lameness indicates that a mild to moderate increase in TNCC due to increase in MNC is associated with macroscopic cartilage damage and microscopic changes in the synovial membrane. All pathological findings were mild and their correlation indicated that these processes are interrelated and that could be ascribed to an early OA.

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Ethical statement

Sample collection was carried out with the approval of the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (number 01-1028).

Authors' contributions

MKF and LM designed the study. LM collected the samples. MR, JFA IM and LM performed the laboratory analyses. MKF performed and interpreted the statistical analyses. AR, MKF, SĐ, IM, MR, LM and JFA interpreted the data and equally contributed in the writing of the manuscript. All authors have read and approved the manuscript.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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POVEZANOST PATOLOŠKIH PROMENA HRSKAVICE, SINOVIJALNE TEČNOSTI I MEMBRANE KOD SRPSKIH PLANINSKIH KONJA BEZ ZNAKOVA HROMOSTI

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Osteoartrtis (OA) kod konja se često razvija na metakarpo/metatarzofalangealnim (MCP/MTP) zglobovima i njegovo otkrivanje u ranoj fazi razvoja je ključno za efikasno lečenje. Pretpostavka je da stepen oštećenja hrskavice pozitivno korelira sa patološkim promenama na sinovijalnoj membrani i u sinovijalnoj tečnosti (SF) MCP/MTP zglobova Srpskih planininskih konja koji su nosili teški teret, ali nisu pokazivali znake hromosti. Studija je sprovedena na 32 MCP/MTP zgloba poreklom od osam konja starosti izmedju sedam i 12 godina. Noge konja su trasportovane sa klanice u obdukcionu salu i uzorkovana je SF. Odredjen je izgled, ukupan broj ćelija sa jedrom (TNCC), broj mononuklearnih ćelija (MNC), ukupni proteini (TP), i haptoglobin (Hp). Uzorci SM za histologiju su uzorkovani sa palmarnog/plantarnog uvrata zglobne kapsule. Makroskopski pregled promena kondilusa treće metakarpalne/metatarzalne kosti sproveden je bojenjem sa Indian Ink tehnikom. Ocenjivanje promena je sprovedeno po preporukama Medjunarodnog društva za istraživanje osteoartritisa .Sinovijalna tečnost je bila bistra, bledo žute boje, umereno viskozna. Polovina uzoraka je imala TNCC iznad, dok su svi imali TP u okviru fizioloških vrednosti. Vrednosti Hp su bile ispod referentog opsega i izostavljene su iz daljih analiza. TNCC je korelirao sa MNC ($\varrho s=0,81$, $P<0,001$), i mikroskopskom ($\varrho s=0,62$, $P=0,003$) i makroskopskom ocenom ($\varrho s=0,47$, $P=0,008$). Pored toga, MNC je korelirao sa makroskopsom ocenom ($\varrho s=0,40$, $P=0,03$). Sve patološke promene su bile umerenog intenziteta i njihova korelacija ukazuje da su ovi procesi međusobno povezani i da se mogu pripisati ranom OA.