Anatomic and histologic features and ultrasonographic appearance of the collateral ligaments of the metacarpophalangeal and metatarsophalangeal joints in cadaveric limbs from horses without lameness

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Objective—To describe the anatomic and histologic features of the collateral ligaments (CLs) of the metacarpophalangeal (MCP) and metatarsophalangeal (MTP) joints in cadaveric limbs obtained from nonlame horses and to compare the histologic findings with the ultrasonographic appearance of the CLs.

Sample—Medial and lateral CLs of the MCP and MTP joints of 28 limbs (16 forelimbs and 12 hind limbs) from 9 adult nonlame horses euthanized for reasons unrelated to the study.

Procedures—26 limbs of 8 horses were examined by ultrasonography immediately after euthanasia. Postmortem gross and histologic examinations were performed for all 28 limbs. Histologic and ultrasonographic images were graded and compared.

Results—Ultrasonographically, the mean ± SD depth and width of the superficial CL were 5.1 ± 0.7 mm and 20.5 ± 1.7 mm, respectively. On histologic examination, only 125 of 319 (39%) specimens obtained from 56 medial and lateral CLs appeared normal. Histopathologic findings varied from mild changes in cellular density and collagen fiber orientation to severe fibrocartilaginous metaplasia. The degree of CL lesion severity increased distally, and the lateral CL was affected more frequently than was the medial CL. Ultrasonographically detectable abnormalities were not correlated with the histologic findings.

Conclusions and Clinical Relevance—In horses, histologic abnormalities within the CLs of the MCP and MTP joints may be an adaptive response to joint hyperextension and compression and might predispose horses to desmopathy and ligament failure in the event of trauma. Ultrasonography did not detect morphologic changes of the CL matrix. For an accurate diagnosis of subclinical lesions, more sensitive imaging techniques (eg, MRI) should be considered. (*Am J Vet Res* 2014;75:1089–1098)

In horses, the MCP and MTP (fetlock) joints are intensely loaded high-motion joints, which require a complex network of stabilizing structures to withstand loading forces.[1,2] The CLs limit the joint motion to the sagittal plane, and have a role in fetlock suspension.[3,4] The CLs of the MCP and MTP joints each consist of 2 portions. The superficial (long) layer extends vertically from the distal third portion of the MCIII or MTIII bone to the proximal extremity of the PP bone. The deep (short) portion is oblique and triangular in shape and radiates from its origin in the ligament fossa of the MCIII or MTIII condyle in a distopalmar or distoplantar direction and attaches on the PP bone and the corresponding PS bone.[3,5,6]

To determine the most frequent problems in a large population of performance horses with lameness localized to the MCP and MTP joint regions, King et al[7] described clinical features and associated MRI findings. Among the 232 horses examined in that study,[7] the prevalence of CL desmitis was approximately 7%

Abbreviations

CL	Collateral ligament
MCP	Metacarpophalangeal
MCIII	Third metacarpal
MTIII	Third metatarsal
MTP	Metatarsophalangeal
PP	Proximal phalangeal
PS	Proximal sesamoid

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(33 horses). Osteochondral defects, intersesamoidean ligament desmitis, annular ligament injury, articular cartilage injury, and superficial and deep digital flexor tendon injury were less common than CL desmitis of the MCP and MTP joints.[7] In another study[8] of horses with lameness, the prevalence of CL desmitis of the MCP and MTP joints identified by means of MRI was similar (3/40 [7.5%] horses) to that reported by King et al. However, the most common technique used to examine the CLs of the MCP and MTP joints of horses in private practice is ultrasonography.[9,10] Ultrasonographic examination of the lateral and medial aspects of the MCP or MTP joint region allows the identification of subcutaneous connective tissues as well as the superficial and deep CLs, which are symmetric laterally and medially. Given that a superficial CL has echogenic fiber bundles parallel to the skin[11] and runs in a proximodistal direction perpendicular to the weight-bearing surface of the MCP or MTP joint, it is easily imaged in the longitudinal plane. In contrast, because a deep CL has an oblique fiber direction and transverses the joint in a dorsoproximal-palmarodistal or dorsoproximal-plantarodistal orientation, it is more difficult to examine ultrasonographically and the scan plane has to be adjusted accordingly. Especially at its origin in the condylar fossa, where the fibers arising at the bone surface bend, the deep CL physiologically appears hypoechogenic, compared with the remainder of the deep CL on frontal and transverse sections.[11]

Although ultrasonography is excellent for soft tissue imaging, it has limitations for identification of lesions.[12] Especially in the CL of the MCP or MTP joint in horses, the heterogeneous appearance of the differently oriented fibers can lead to misinterpretation of ultrasonographic results.[9,13–15]

To the authors' knowledge, the correlation between ultrasonographic and histopathologic findings for the CLs of the MCP and MTP joints in horses has not been reported. The objective of the study reported here was to describe the anatomic and histologic features of the CLs of the MCP and MTP joints in cadaveric limbs obtained from nonlame horses and to compare those findings with the ultrasonographic appearance of the CLs. Of interest was whether the relatively low incidence of clinically or ultrasonographically evident CL injuries other than ruptures correlated with histopathologic findings.

Materials and Methods

Horses—Nine adult clinically nonlame horses (4 mares, 3 stallions, and 2 geldings) with no palpable abnormalities of the fetlock regions were used for the study. Mean ± SD age of the horses was 13 ± 8 years (range, 3 to 28 years); mean weight was 521 ± 85 kg. Breeds were warmblood (n = 2), Hanoverian (1), Oldenburg (1), Selle Francais (1), Thoroughbred (1), Trotter (1), Friesian (1), and Haflinger (1). The disciplines in which they were used included pleasure (n = 3), show-jumping (2), racing (1), breeding (1), and fiaker driving (1). The use of 1 horse was unknown.

Horses underwent euthanasia by slow IV injection of embutramide, mebezonium iodide, and tetracaine hydrochloride solution at the University of Veterinary Medicine Vienna from July to November 2012 for reasons unrelated to this study. Twenty-eight limbs (16 forelimbs and 12 hind limbs) were used in the study. From 5 horses all 4 limbs were used, from 3 horses only the forelimbs were used, and from 1 horse only the hind limbs were used. All procedures were performed in compliance with the guidelines of the Ethics Committee of the Vetmeduni Vienna.

Ultrasonography—The superficial and deep portions of the lateral and medial CLs of 26 limbs (14 forelimbs and 12 hind limbs) of 8 horses were examined in the longitudinal and transverse planes with an ultrasound machine[a] equipped with a 12-MHz linear transducer. Two forelimbs of 1 horse were not available for ultrasonographic examination owing to logistical reasons. All ultrasonographic examinations were carried out within 3 hours after euthanasia by the same experienced ultrasonographer (JE). Limbs were transected through the middle carpal or talocalcaneal-centroquartal joint and mounted in a custom-built fixture to imitate the physiologic hyperextended dorsal angle of 135° to 140° of a weight-bearing limb. The MCP or MTP joint region was clipped, and the skin was soaked with lukewarm water and coated with coupling gel. During each examination, the gain settings and the focus were adjusted to optimally define the CLs. Transverse and longitudinal digital still images of the CLs were obtained throughout their length; depth and width were measured and graded at the end of the completed examination. All measurements were made by a single operator (JE). For grading purposes, all images (transverse and longitudinal) were reviewed by 2 authors (JE and FJ) independently of each other.

Because the deep CL and the superficial CL had a similar ultrasonographic appearance in all fetlocks examined during the study and because images of the superficial CL are more easily and reliably obtained than are images of the obliquely oriented deep CL, we selected the region of the superficial CL where it crosses the MCP or MTP joint as the most representative aspect of the CLs with reproducible landmarks for ultrasonographic and comparative histologic scoring. Each superficial CL was scored on the basis of its echogenicity (scale from 0 to 4 as described previously,[5,16,17] where 0 = normal, 1 = slightly hypoechoic, 2 = 50% anechoic, 3 = mostly anechoic, and 4 = completely anechoic) and its fiber alignment (scale from 0 to 3 as described previously,[5,16,17] where 0 = > 76% parallel linear echoes, 1 = 51% to 75% parallel linear echoes, 2 = 26% to 50% parallel linear echoes, and 3 = 0% to 25% parallel linear echoes). A combined grade was calculated by adding the echogenicity and the fiber alignment scores and dividing the sum by 2. A final combined grade for each medial or lateral superficial CL was then determined by adding the combined grade for each reviewer and dividing the sum by 2.

Anatomic features—After ultrasonography, dissection of all limbs was performed on the same day (20 limbs) or the next day (8 limbs). If dissection was performed on the next day, the limb was cooled at 4°C (39.2°F) overnight (approx 12 hours). A dorsal sagittal incision was made 1 cm proximal to the coronary band

and extended vertically to the middle third of the MCIII or MTIII bone. The distal and proximal ends of the incision were extended horizontally to the level of the superficial digital flexor tendon. The skin, the extensor branch of the interosseus medius muscle, and the fascia were removed to expose the CL.

Histologic examination—The CLs of the MCP and MTP joints were resected and fixed in neutral-buffered 4% formalin for at least 4 days (mean ± SD immersion time, 10 ± 7 days; range, 4 to 21 days). Each of the 56 medial and lateral CLs of the MCP and MTP joints was divided into 6 parts (4 superficial and 2 deep parts) as follows: the origin of the superficial CL, proximal portion of the superficial CL body, distal portion of the superficial CL body, insertion of the superficial CL, origin of the deep CL, and insertion of the deep CL (**Figure 1**). In 1 horse, only the superficial part of the medial and lateral CL of both forelimbs were available for the histologic examination. Thus, 328 tissue samples (184 from the MCP joint and 144 from the MTP joint) were obtained for examination. Tissue samples were embedded longitudinally in paraffin using a 24-hour prolonged embedding protocol. Subsequently, specimens were sectioned sagittally at 4-µm intervals with a rotary microtome[b] and stained with H&E, safranin O, Alcian blue, or van Gieson stain.

All sections were evaluated by the same person (FP) on the basis of a modified version of a previously reported histologic grading scale,[18] where 0 = apparently normal, 1 = variations in cellular shape and density with variations in collagen fiber structure and orientation, 2 = presence of chondroid cells indicative of moderate fibrocartilaginous metaplasia, and 3 = severe fibrocartilaginous metaplasia. Each histologic sample was evaluated separately. In particular, we compared the occurrence of variations between the medial and lateral and the superficial and deep CL. Finally, the morphology of the 2 specimens representing the superficial CL body (Figure 1) was separately compared with its ultrasonographic appearance.

Statistical analysis—Owing to the limited sample size and the level of measurement of the histologic grades (ordinal scale), raw grades for each histologic sample were used for further analysis. For statistical analysis,[c] a χ^2 test was used to compare histologic grades for the medial and lateral CLs. Significance was set at a value of $P < 0.01$. A Spearman-rho correlation test was used to detect an association between histologic or ultrasonographic grades and CL measurements.

Results

Ultrasonographic examination findings—With regard to the echogenicity and fiber alignment, the ultrasonographic appearance of the superficial and deep CL of each joint was similar in all limbs examined in the study. Likewise, the ultrasonographic changes, where present, were distributed diffusely throughout the length of the CL, with no predilection for localization at the origin, body, or insertion.

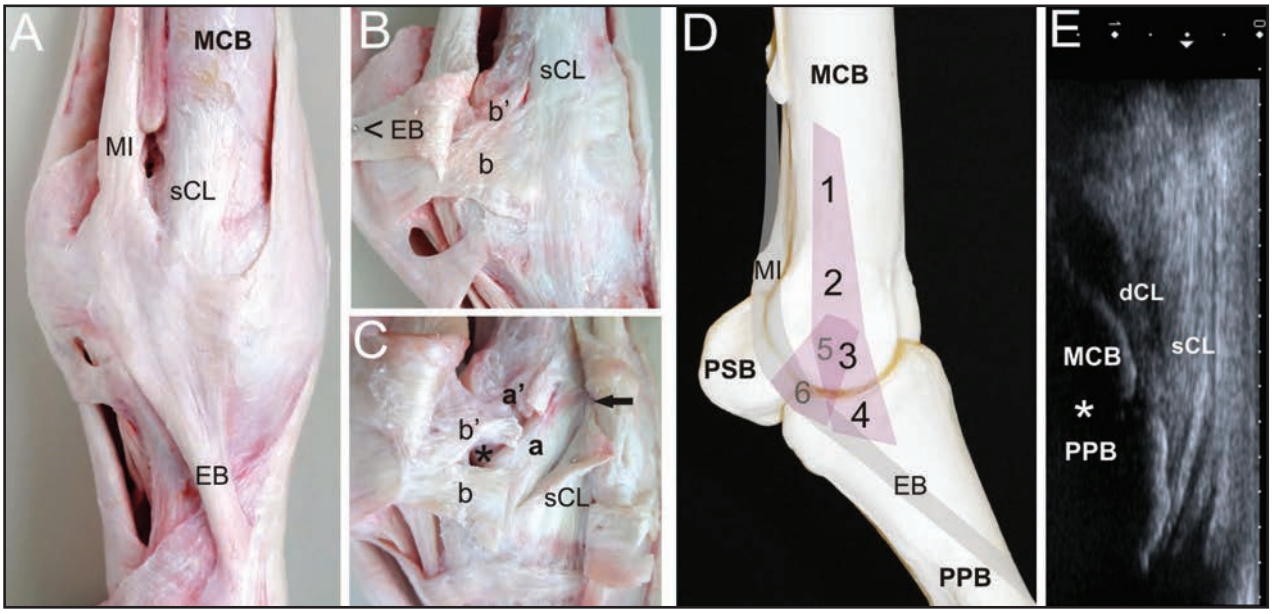

Figure 1—Representative photographs of the topographic (A) and anatomic (B and C) features, schematic view (D), and an ultrasonographic image (E) of the CLs of the MCP and MTP joints in cadaveric equine limbs. A—Photograph of an MCP joint. The lateral CL is located under the dorsal extensor branch (EB) of the interosseus medius muscle (MI), which extends to the common extensor tendon. MCB = Third metacarpal bone. sCL = Superficial CL. B—Photograph of the same MCP joint in panel A. After removal of the EB (arrowhead) of the MI, the CL of the PS bone is visible. b = Branch of the CL of the PS bone to the PP bone. b' = Branch of the CL of the PS bone to the MCIII bone. C—Photograph of the same MCP joint in panels A and B. After transection of the sCL (arrow) and transection of the sesamoidean CL (b, b'), 2 parts of the deep CL (a = branch to the PP bone; a' = branch to the PS bone) and the joint space (asterisk) are visible. D—Schematic view of the 6 parts of resected CLs of equine MCP and MTP joints from which specimens were collected for histologic examination as follows: 1, origin of the superficial CL; 2, proximal portion of the superficial CL body; 3, distal portion of the superficial CL body; 4, insertion of the superficial CL; 5, origin of the deep CL; and 6, insertion of the deep CL. PSB = Proximal sesamoid bone. PPB = Proximal phalangeal bone. E—Representative ultrasonographic image illustrating the longitudinal view of the sCL and deep CL (dCL) of equine MCP (and MTP) joints. The location of this image is where the right lateral sCL and dCL cross the joint space (asterisk) between the MCIII bone (MCB) and the PPB.

Table 1—Ultrasonographic grades assigned to medial and lateral superficial CLs of the MCP and MTP joints in 26 cadaveric equine limbs (14 forelimbs and 12 hind limbs from 8 horses).

	Forelimb				Hind limb			
	Left		Right		Left		Right	
Horse	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial
1	2	2	0	1	1	0	0	0
2	0	1	2	0	1	0	1	0
3	0	0	1	0	0	0	0	0
4	1	1	1	0	0	0	0	1
5	0	0	0	0	—	—	—	—
6	0	0	0	0	1	1	0	0
7	—	—	—	—	0	0	0	0
8	0	1	2	2	—	—	—	—

Ultrasonographic examinations were carried out within 3 hours after euthanasia. The region of the superficial CL where it crosses the MCP or MTP joint was considered the most representative aspect of the CLs with reproducible landmarks for ultrasonographic (and comparative histologic) grading. Transverse and longitudinal digital still images of the CLs were obtained throughout their length; for grading purposes, all images (transverse and longitudinal) were reviewed by 2 investigators independently of each other. Each superficial CL was scored on the basis of its echogenicity (scale from 0 to 4, where 0 = normal, 1 = slightly hypoechoic, 2 = 50% anechoic, 3 = mostly anechoic, and 4 = completely anechoic) and its fiber alignment (scale from 0 to 3 as described previously, where 0 = > 76% parallel linear echoes, 1 = 51% to 75% parallel linear echoes, 2 = 26% to 50% parallel linear echoes, and 3 = 0% to 25% parallel linear echoes). A combined grade was calculated by adding the echogenicity and the fiber alignment scores and dividing the sum by 2. A final combined grade for each medial or lateral superficial CL was then determined by adding the combined grade for each reviewer and dividing the sum by 2.

— = No data available.

Ultrasonographically, the depth of the superficial CL in the 14 MCP (14 medial and 14 lateral CLs) and 12 MTP (12 medial and 12 lateral CLs) joints ranged from 3.8 to 6.5 mm (mean ± SD, 5.1 ± 0.7 mm) and the width ranged from 17.2 to 23.1 mm (mean ± SD, 20.5 ± 1.7 mm). Among the 26 lateral superficial CLs, 16 had an ultrasonographic grade of 0, 7 had an ultrasonographic grade of 1, 3 had an ultrasonographic grade of 2, and none had an ultrasonographic grade of 3 (**Table 1**). Among the 26 medial superficial CLs, 18 had an ultrasonographic grade of 0, 6 had an ultrasonographic grade of 1, 2 had an ultrasonographic grade of 2, and none had an ultrasonographic grade of 3. Two horses had grades > 0 concurrently for the medial and lateral CLs of 1 forelimb; 4 horses had grades > 0 concurrently in more than 1 limb.

Anatomic features—No gross alterations were observed on dissection of the MCP and MTP joints investigated. The extensor branches of the interosseus medius muscle extend dorsolaterally and dorsomedially to the common (or long) digital extensor tendon at the abaxial aspect of the PP bone and cross the distal part of the medial and lateral CL of the MCP or MTP joint. The medial or lateral CL itself consists of a long and thin superficial layer and a short, more substantial deep layer.

The medial or lateral superficial CL originates medially or laterally from the distal quarter of the MCIII or MTIII bone with the enthesis extending from the level of the distal end of the second and fourth metacarpal or metatarsal bones to the eminences of the distal aspect of the MCIII or MTIII bone. The superficial CL then spans the joint in a vertical direction and inserts just distal to the joint margin on the proximolateral or proximomedial aspect of the PP bone. Thus, the proximal portion of the superficial CL seems comparatively long, whereas the distal portion is short (Figure 1).

The medial or lateral deep CL has an oblique orientation and a triangular shape. It begins in the epicondylar fossa of the MCIII or MTIII bone. In the forelimb, it runs in a distopalmar direction to the palmarolateral or palmaromedial surface of the PP bone and the abaxial margin of the adjacent PS bone (Figure 1). Each deep CL is crossed by the collateral sesamoidean ligament, which also inserts at the epicondylar fossa of the MCIII bone and the palmarolateral or palmaromedial aspect of the PP bone originating at the PS bone. In the hind limb, the anatomic features of the medial or lateral deep CL are similar to those of the medial or lateral deep CL in the forelimb.

Histologic examination findings—Nine of 328 CL specimens from the MTP joints of 3 horses (1 lateral and 3 medial CL) and from the MCP joints of 2 horses (2 lateral and 3 medial CL) had to be excluded from the study because of severe processing artifacts. Only 125 of 319 (39%) specimens were considered normal (histologic grade 0), being characterized by elongated cells embedded in a collagen-rich extracellular matrix and organized longitudinally to the long axis (**Figure 2**).

Among the 319 specimens, 120 (38%) were assigned a histologic grade of 1. Sections of these specimens contained hypocellular areas with loss of fiber orientation and a decrease of overall collagen fiber density, compared with normal tissue. Concurrent hypercellular areas were characterized by cells with rounded nuclei, which were aligned in a row or accumulated in small clusters (Figure 2).

Seventy-four of the 319 (23%) specimens had fibrocartilaginous metaplasia; the metaplasia in 47 (63.5% of the fibrocartilaginous specimens [15% of all specimens]) was moderate (histologic grade 2). Rounded or chondroid cells were arranged in pairs, small clusters, or longitudinal rows and were surrounded by a lacunar space of extracellular matrix. Collagen fibers were separated by greater than normal amounts of basophilic mucoid ground substance, which was confirmed by van Gieson, Alcian blue, and safranin O staining (Figure 2).

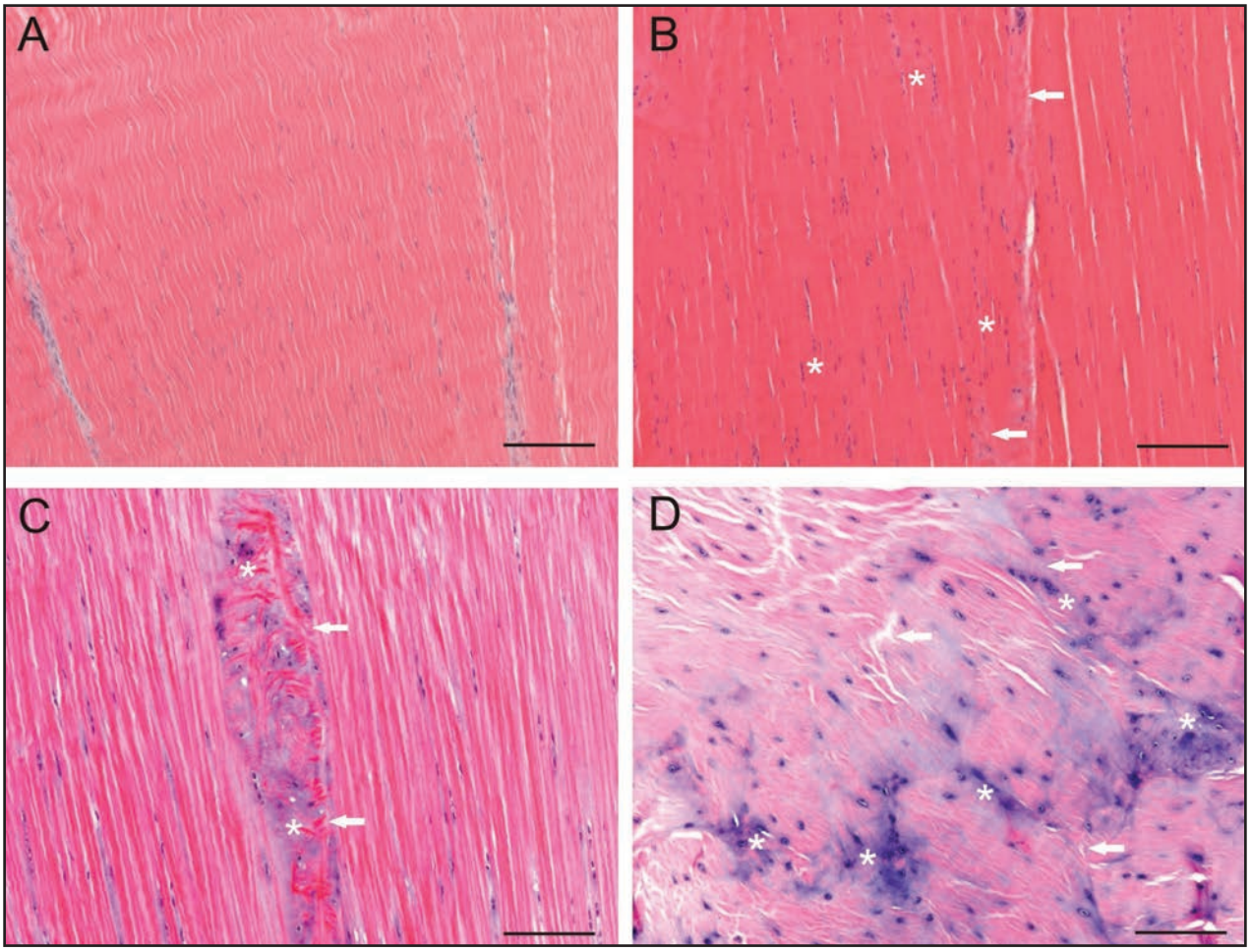

Figure 2—Representative photomicrographs of longitudinal sections of the midbody of the superficial CL of the lateral MTP joints (A and B) and lateral MCP (C and D) joints in cadaveric equine limbs. All sections were evaluated and assigned a histologic grade (0 through 3, where 0 = normal and 3 = severe fibrocartilaginous metaplasia). A—Section in which linearly arranged collagen fibers are interspersed with elongated cells (tissue was assigned a histologic grade of 0). B—Section in which there is mild disorganization of collagen fibers (arrows) and variation in cellular density; some areas of higher cell density (asterisks) are visible (tissue was assigned a histologic grade of 1). C—Section in which chondroid cells are present, indicative of moderate fibrocartilaginous metaplasia (asterisks), with loss of fiber orientation (arrows; tissue was assigned a histologic grade of 2). D—Section in which basophilic hyaline cartilage-like areas are visible, indicative of severe fibrocartilaginous metaplasia (asterisks), with severe loss of fiber orientation (arrows; tissue was assigned a histologic grade of 3). H&E stain; bar = 100 µm.

Severe changes were detected in 27 specimens (36.5% of the fibrocartilaginous specimens [8% of all specimens]; Figure 2). The fibrocartilaginous tissue of these specimens contained small hyaline cartilage-like areas, which were characterized by a homogenous basophilic matrix with scattered plump chondroid cells. Safranin O and Alcian blue staining confirmed the presence of greater than normal amounts of mucoid ground substance and cartilage in sections of each of those specimens (**Figure 3**).

Overall, all horses included in the study had histologic changes in at least 1 part of either the superficial or deep CL of a MCP or MTP joint (**Figure 4**). In some instances, histologic grades differed among different parts within 1 ligament. The origin of the superficial CL was the only ligament part wherein fibrocartilaginous changes were not detected. Within the other ligament parts, the histologic grade and incidence of lesions increased distally; at the distal insertion of the deep CL, the histologic abnormalities were most severe. In particular, 43 specimens of the proximal superficial CL were graded as 0, but only 15 specimens of the distal superficial CL and 2 specimens of the distal deep CL were evaluated to be normal, whereas 14 specimens of the distal deep CL were scored with a grade of 3.

When the histologic grades for each part of the medial and lateral CLs were compared, a significantly (P = 0.005) higher frequency of more severe lesions was found in the lateral CL. Forty-nine specimens of the lateral CL had moderate to severe (grades 2 and 3) fibrocartilaginous changes, whereas only 25 specimens of the medial CL were at least moderately affected (**Figure 5**).

Comparison of ultrasonographic and histologic examination findings—The histologic sections of the proximal and distal portions of the superficial CL body were compared with their ultrasonographic appearance (Figure 1). Because 1 horse (2 limbs; 8 histologic specimens) did not undergo an ultrasonographic examination and 2 histologic specimens of the superficial CL body from 2 horses were excluded

from the study owing to severe processing artifacts, 102 specimens met the criteria for comparison. Seventeen of the 102 specimens had fibrocartilaginous changes (histologic grade ≥ 2; **Table 2**). Of these 17 specimens, 12 had no detectable ultrasonographic abnormalities (**Figure 6**), 4 had an ultrasonographic

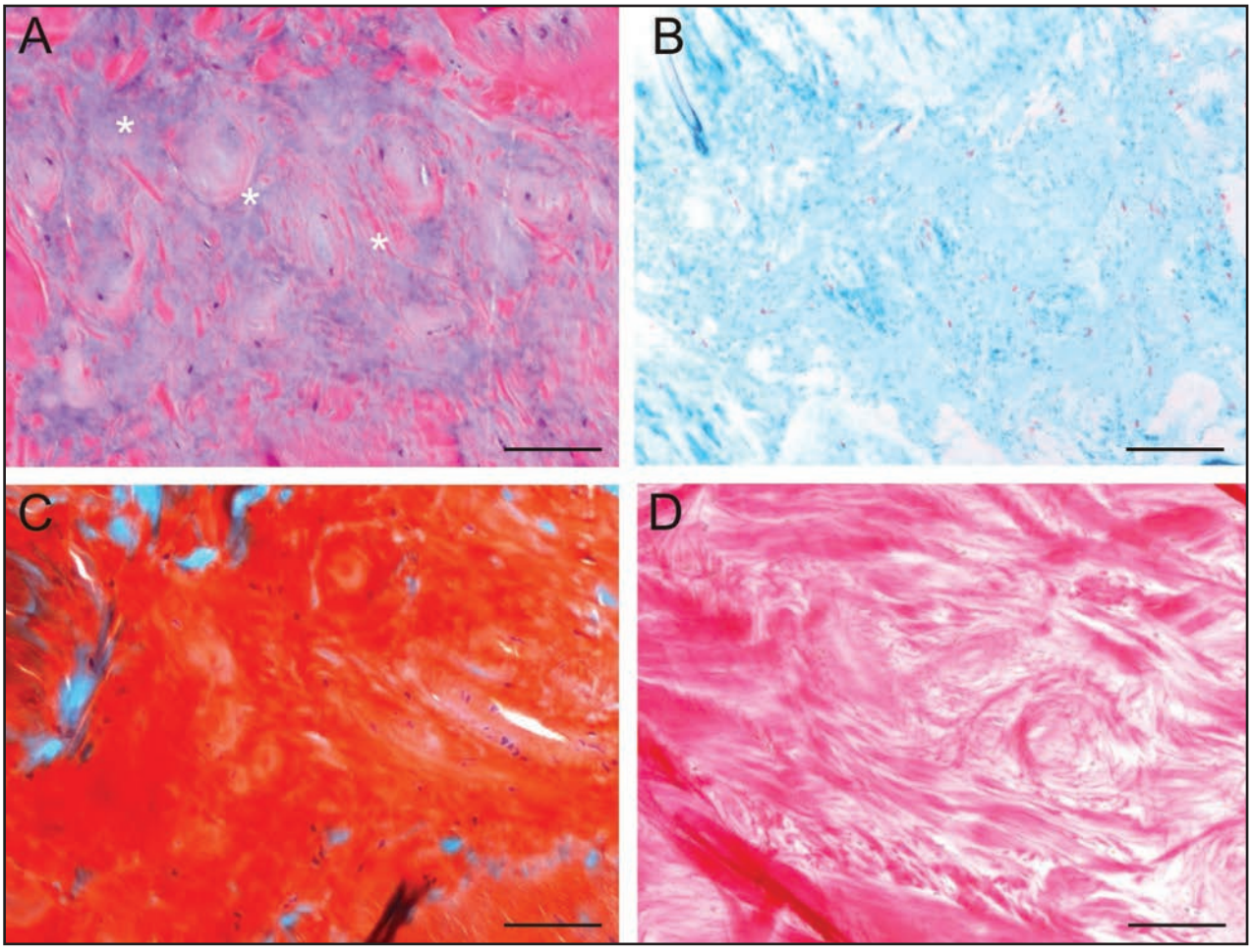

Figure 3—Representative photomicrographs of longitudinal sections of the midbody of the left medial deep CL of the MCP joint in a cadaveric equine limb of 1 horse. All sections were evaluated and assigned a histologic grade of 3 (severe fibrocartilaginous metaplasia). A—Section stained with H&E stain. Notice the hypocellularity (asterisks) of the tissue with a greater than normal amount of mucoid ground substance (areas stained blue). B—Section stained with Alcian blue stain. The large amount of mucoid ground substance is evident by the Alcian blue stain uptake. C—Section stained with safranin O stain. Orange-stained areas represent mucoid ground substance. D—Section stained with van Gieson stain. Severe loss of fiber orientation is revealed following van Gieson staining. Bar = 100 µm.

Table 2—Ultrasonographic and histologic grades for the superficial CL body of the MCP and MTP joints in the cadaveric equine limbs in Table 1.

Histologic grade	Ultrasonographic grade				Total No. of samples
	0	1	2	3	
0	28	13	4	0	**45**
1	29	8	3	0	**40**
2	10	3	1	0	**14**
3	2	1	0	0	**3**
Total No. of samples	**69**	**25**	**8**	**0**	**102**

Data represent the number of tissue samples for histologic assessment, each of the 56 medial and lateral CLs of the MCP and MTP joints was divided into 6 parts (4 superficial and 2 deep parts) as follows: the origin of the superficial CL, proximal portion of the superficial CL body, distal portion of the superficial CL body, insertion of the superficial CL, origin of the deep CL, and insertion of the deep CL. For each specimen, a histologic grade was assigned where 0 = normal, 1 = variations in cellular shape and density with variations in collagen fiber structure and orientation, 2 = presence of chondroid cells indicative of moderate fibrocartilaginous metaplasia, and 3 = severe fibrocartilaginous metaplasia. Because 1 horse (2 limbs) did not undergo ultrasonographic examination, and 2 histologic specimens of the superficial ligament body were excluded from the study owing to severe processing artifacts, a total of 102 samples met the criteria for comparison. The histologic grade of both the proximal and distal portion of the superficial ligament body was used for comparison; therefore, the ultrasonographic grade was used twice.

See Table 1 for key.

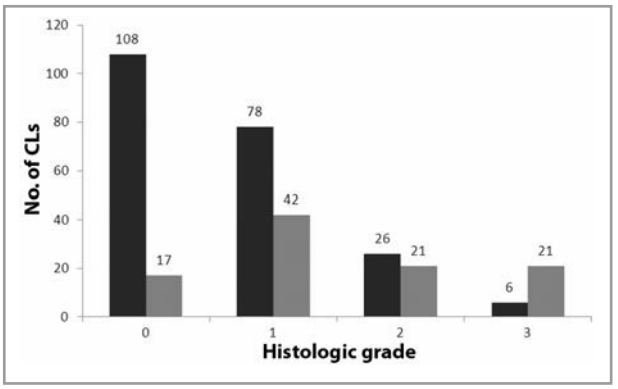

Figure 4—Distribution of histologic grades among specimens of medial and lateral superficial (black bars) and deep (gray bars) CLs of the MCP and MTP joints obtained from 28 cadaveric equine limbs (16 forelimbs and 12 hind limbs from 9 horses). Each of the 56 medial and lateral CLs of the MCP and MTP joints was divided into 6 parts (4 superficial and 2 deep parts) as follows: the origin of the superficial CL, proximal portion of the superficial CL body, distal portion of the superficial CL body, insertion of the superficial CL, origin of the deep CL, and insertion of the deep CL. In 1 horse, only the superficial part of the medial and lateral CL of both MCP joints was available for histologic examination. Of the 328 tissue samples obtained for examination, 9 had severe processing artifacts; thus, 319 CL specimens were assessed (179 specimens of both CLs of 16 MCP joints and 140 specimens of 12 MTP joints of 9 horses). Values above each bar represent the actual number of specimens.

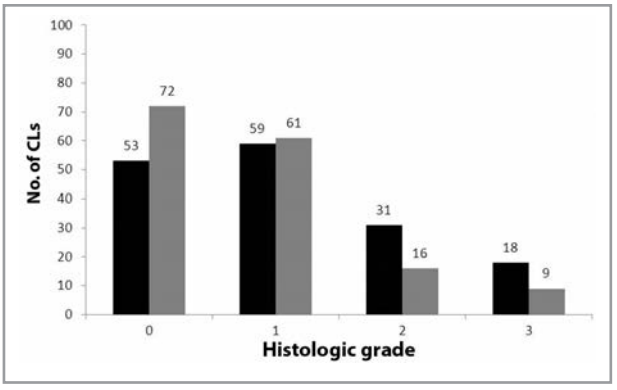

Figure 5—Distribution of histologic grades among specimens of lateral (black bars) and medial (gray bars) CLs of the MCP and MTP joints obtained from 28 cadaveric equine limbs (16 forelimbs and 12 hind limbs from 9 horses). Each ligament was divided in 6 parts. In 1 horse, only the superficial part of the medial and lateral CL of both MCP joints was available for histologic examination, which resulted in 4 specimens each. Of the 328 tissue samples obtained for examination, 9 had severe processing artifacts; thus, 319 CL specimens were assessed (179 specimens of both CLs of 16 MCP joints and 140 specimens of 12 MTP joints of 9 horses). Each histologic sample was evaluated separately, and the occurrences of variations were compared between medial and lateral CLs. Values above each bar represent the actual number of specimens. *See* Figure 4 for key.

grade of 1, and 1 had an ultrasonographic grade of 2. Of the remaining 85 specimens with a histologic grade of 0 or 1, 57 had an ultrasonographic grade of 0, 21 had an ultrasonographic grade of 1, and 7 had an ultrasonographic grade of 2. Although specimens with a histologic grade of 0 or 1 were often obtained from superficial CL bodies that had a normal ultrasonographic appearance, there was no significant correlation between ultrasonographic and histologic grades for the superficial CL body.

Discussion

In the present study, the anatomic and histologic features of the CLs of the MCP and MTP joints in cadaveric limbs obtained from nonlame horses were investigated, and the histologic findings were compared with the ultrasonographic appearance of the CLs. Results indicated that there were differences between histologic and ultrasonographic examination findings, and the correlation between histologic and ultrasonographic grades for the CLs was poor. In 7 of 85 CL specimens for which histopathologic findings unremarkable to mild, the ultrasonographic grade was 2. Although this is a small proportion of specimens, false positive ultrasonographic findings could have serious detrimental consequences for clinical patients. There are various explanations for these false positive ultrasonographic findings, of which presence of ultrasonographic artifacts is certainly among the foremost. In the present study, we attempted to limit the factors that might possibly contribute to artifact formation by clipping the hair on each limb, applying copious amounts of a coupling gel, adjusting the gain settings and the focus to optimally define the CLs, and by having only 1 ultrasonographer perform all examinations to limit operator error. Because fiber orientation varies between the superficial and deep portion as well as at the point of origin and insertion of each CL, frequent adjustments of the probe tilt were made to attain an angle of incidence of 90° to the imaged structure; the probe tilt was altered when the CLs appeared hypoechoic to optimize the angle of incidence for the specific portion of the CL under examination to exclude an artifact. Other factors that could have contributed to false-positive ultrasonographic findings include probe-skin artifacts (caused by inadequate contact of the probe faceplate with the skin owing to size or shape mismatch between the footprint of the probe and the anatomic region examined or by incorrect position of the probe on the skin)[19] and scattering because the ultrasound beam in the fetlock region cannot always be positioned perpendicular to the tissue interface over the entire surface of the transducer because of tissue geometry. Use of a higher-frequency ultrasound probe with a smaller footprint might have reduced the likelihood of probe-skin artifacts and improved axial and lateral resolution. In addition, use of a stand-off pad could have increased the contact area and therefore possibly enhanced image quality. The 12-MHz linear array transducer used in the present study was well within the recommended probe frequency range for ultrasonographic examinations of the distal portions of horses' limbs. Ultrasonographic findings for the CLs should therefore generally be interpreted cautiously and ideally put in context with further complementary diagnostics.

In 12 of 17 specimens of the superficial CL body that had moderate to severe fibrocartilaginous metaplasia in the present study, no ultrasonographic abnormalities were observed. These findings indicated that ultrasonography might not be sufficiently sensitive for detection of subclinical morphologic changes within the CLs of the MCP or MTP joint in horses. Therefore, it would be interesting to evaluate the CLs of nonlame horses by means of MRI and assess the

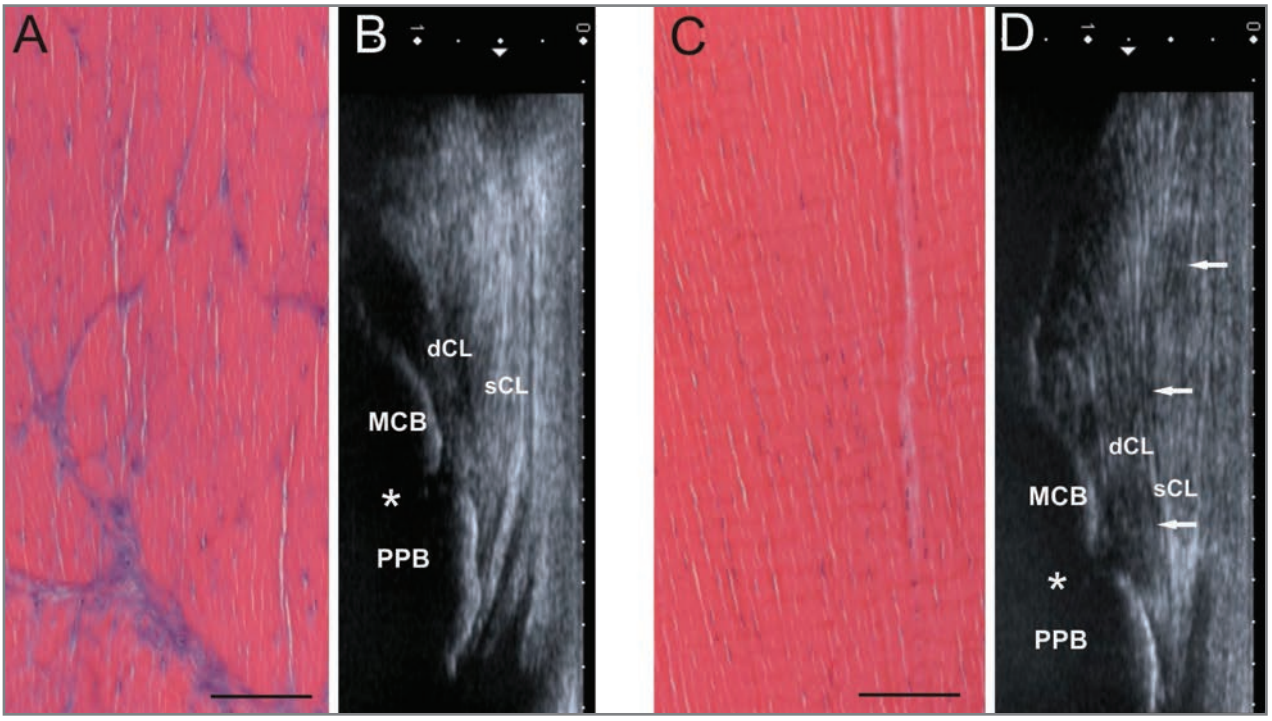

Figure 6—Photomicrographs (A and C) of longitudinal sections of tissue from the body of the superficial CL ligament of the MCP joint in cadaveric limbs from 2 horses with the corresponding longitudinal ultrasonographic images (B and D) of each ligament obtained within 3 hours after euthanasia. A and B—Tissue section and ultrasonographic image of a lateral superficial CL body from the right MCP joint of 1 horse. Notice the fibrocartilaginous modifications (tissue was assigned a histologic grade of 3). However, the ultrasonographic appearance was considered normal (grade 0); the sCL and dCL appeared to have no abnormalities. C and D—Tissue section and ultrasonographic image of a medial superficial CL body from the left MCP joint of another horse. Microscopically, the ligament had no abnormalities (tissue was assigned a histologic grade of 0). Nevertheless, the ultrasonographic grade assigned for this ligament was 2; the sCL and dCL were thickened and hypoechoic with partial loss of the parallel fiber alignment (evident ultrasonographically as partial loss of parallel linear echoes) and disorganization of fibers (arrows). In both ultrasonographic images, the joint space between the MCIII bone and the PPB is indicated (asterisk). For tissue sections, H&E stain; bar = 100 µm. *See* Figure 1 for remainder of key.

correlation between MRI and histologic examination findings.

Several reports[3,5,11,20–23] describe the normal anatomic features of the equine MCP and MTP joints and their CLs. Although Wissdorf et al[20] stated that the deep CL attaches at the PP bone only, Barone[21] specified that it inserts by blending its fibers with those of the collateral sesamoidean ligament. In contrast, Dyce et al[22] reported that the MCP or MTP joint CLs connect the MCIII or MTIII with the PP bone and an additional smaller and triangular CL anchors the PS bone to the sides of the MCIII or MTIII condyle and the proximal tubercles of the PP bone. Accordingly, we found that the CLs of the MCP or MTP joints consisted of 2 parts. The superficial layer was long and ran vertically from the distal aspect of the MCIII or MTIII bone to the PP bone. The deep portion was triangular and oblique and extended from the ligament excavation of the MCIII or MTIII condyle to the PP bone and the abaxial margin of the PS bone. This anatomic description agrees with reports of other studies[3,5,6,11,24,25] in which the anatomic features and results of imaging of the equine MCP and MTP regions were investigated.

Histologic examination of specimens of CLs in the present study revealed that normal ligamentous tissue was predominantly composed of parallel collagen fibers with sparsely interspersed ligament cells, as described in many other reports.[26–30] The grades used to characterize histopathologic findings were assigned by use of a modified version of a scoring system devised by Dyson et al,[18] who studied the CLs of the distal interphalangeal joint in cadaveric limbs from lame and nonlame horses. The scoring system had to be adapted for our study purposes because the original grade 3 was characterized by extensive fissuring degeneration (potentially reflecting previous injury), which was not present in the specimens that we examined or in the group of nonlame horses in their study.[18] However, we found that early alterations of cell shape and fiber alignment indicated a separate stage of adaptation (grade 1) before the first signs of chondroid metaplasia appeared (grade 2). Therefore, we used a more refined grading scale.

Surprisingly, despite inclusion of limbs from nonlame horses in the present study, many CL specimens had histologic evidence of localized collagen fiber irregularities. Possible causes for these changes included microtrauma by a sudden, uneven placement of the foot or chronically imbalanced feet.[31,32] Experimentally induced isolated fiber damage has been shown to result in local loss of homeostatic strain and leads to further extracellular matrix degradation and alteration of the cell-matrix interactions.[31,33] Although the ultimate tensile strength of the tissue is thought to remain unaffected, desmopathy may result if no adequate cellular response occurs.[31] Typically, fibrocartilaginous metaplasia develops in ligaments and tendons subjected to compression.[34–37] It increases the resistance to compression, but reduces the tensile strength of the ligament.[34,35]

Interestingly, the distal third of the superficial and deep CLs, which may be exposed to compressive forces by the extensor branch of the interosseus medius muscle during weight bearing, had the most severe histologic abnormalities in the present study. It is still unclear to what extent the fibrocartilaginous metaplasia within this area is an adaptive process or pathologic degeneration. However, the changes may predispose the CLs to ligament failure.[34,35] Furthermore, we found that the lateral CL was more commonly affected than the medial CL. These histologic findings in nonlame horses are in accordance with the reported higher frequency of injury distribution in the lateral CL of horses.[4,7]

The present study was limited mainly by the small number of and heterogeneity among the available horses. Moreover, histologic data were obtained but not compared with the ultrasonographic appearance in 1 horse (both forelimbs) owing to logistical reasons. Given that some ligament abnormalities are likely to be age related, further studies involving age-matched horses are recommended. Lameness examinations were outside the scope of the present study; thus, the clinical relevance of the study findings remains to be evaluated.

Nevertheless, the results of the present study indicated that degenerative abnormalities in the CLs of the MCP and MTP joints in horses are fairly common because of 319 CL specimens obtained from 28 cadaveric equine limbs (16 forelimbs and 12 hind limbs; 179 specimens of both CLs of 16 MCP joints and 140 specimens of 12 MTP joints from 9 horses), only 125 appeared normal on histologic examination. Changes vary from mild changes in cellular density and collagen fiber orientation to severe fibrocartilaginous metaplasia. These changes might represent adaptive responses to the stress that these joints undergo during locomotion and may predispose horses to lameness. The correlation between histologic and ultrasonographic examination findings was poor, and it was concluded that ultrasonography has limitations in identifying subclinical lesions within the CLs of MCP and MTP joints in horses.

a. Aplio XG, Toshiba, Tokyo, Japan.
b. MICROM HM 355, Thermo Fisher Scientific Inc, Kalamazoo, Mich.
c. IBM SPSS Statistics, version 20.0, IBM SPSS Inc, Armonk, NY.

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