Injury to the suspensory ligament (SL) of horses can be a career-ending injury across a multitude of disciplines. Injuries to the SL, both in the forelimbs and the hind limbs, are typically discussed with reference to 3 regions. These regions are the proximal one-third (origin), the middle one-third (body), and the distal one-third, from approximately where the ligament begins to branch down to the insertion of the branches on the proximal sesamoid bones (branches). In sport horses, the most frequently injured of the 3 regions is the proximal region (origin), and hind limb proximal suspensory injury is significantly more common in comparison to forelimbs.

While suspensory body injuries tend to be seen most frequently in racehorses and branch lesions in race and sport horses, proximal suspensory injury or desmitis tends to be seen across a multitude of breeds and disciplines, including dressage and western performance horses. Additionally, proximal suspensory desmitis is more likely to be bilateral in the hind limbs than in the forelimbs. While proximal suspensory disease in the forelimb is associated with a good prognosis, proximal suspensory injury or desmitis in the hind limbs is associated with rates of return to full athleticism as low as 13% and a high rate of recurrence of lameness following conservative management. Hind limb proximal suspensory desmitis has a significant impact on the horse industry due to its tendency to result in chronic, recurrent, performance-limiting lameness. As a result, understanding the microvasculature of the suspensory ligament is crucial for developing effective treatment strategies.

Microvasculature of the suspensory ligament of the equine hind limb

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OBJECTIVE
To describe the microvascular anatomy of the equine hind limb suspensory ligament.

ANIMALS
18 hind limbs harvested from 9 adult horses euthanized for reasons unrelated to lameness.

METHODS
A catheter was placed in the transected cranial tibial artery at the level of the mid-distal tibia for each hind limb and used to inject 120 to 150 mL of contrast medium (2 limbs) to identify principal vasculature using contrast-enhanced CT or India ink (11 limbs) to identify microvasculature using the Spalteholz tissue-clearing technique. Routine histologic evaluation was performed on transverse sections from 4 hind limbs.

RESULTS
The hind limb suspensory ligament is principally supplied by branches of the medial and lateral plantar metatarsal arteries and, to a lesser extent, the medial and lateral plantar arteries as well as the associated proximal and distal deep plantar arches. A uniformly distributed intraligamentous microvascular supply was observed without relative deficiencies in vascularity between the proximal, midbody, and distal regions. Histologic examination supported these findings, demonstrating a network of connective tissue surrounding and entering the suspensory ligament containing cross-sections of branches of the principal vasculature.

CLINICAL RELEVANCE
The equine hind limb suspensory ligament has a uniformly distributed and abundant microvascular supply throughout its length, with no evidence of relative deficiency of vascular supply in any region. A region of hypovascularity does not appear to be a viable explanation for the high rate of injury to and commonality of lameness associated with the proximal hind suspensory ligament in horses.

Keywords: equine, suspensory ligament, hind limb, microvasculature, anatomy

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result, numerous treatment strategies have been investigated to promote neovascularization and improve the healing potential, prognosis, and long-term soundness potential of these horses. These include the use of more conservative therapies, such as shockwave therapy,11–14 high-intensity laser therapy,15,16 or injection with biologic agents such as platelet-rich plasma,17,18 as well as surgical procedures, including microfracture, ligament splitting, and fasciotomy with neurectomy of the deep branch of the lateral plantar nerve.23–25

There is a body of literature that supports anatomic study of the microvasculature of tendons and ligaments in human medicine. Hypovascular regions of the fibrocartilage of the wrist and posterior tibial tendon have been suggested as an explanation for differences in patterns of injury and wound healing within these structures.22,23 Similarly, decreased microvasculature of the supraspinatus tendon may increase the risk of rotator cuff injury in humans.24 In horses, microvascular anatomy of the superficial digital flexor tendon, deep digital flexor tendon, and SL of the forelimb have been described. In these studies, areas with relatively deficient or absent vasculature were identified that correlated with areas prone to injury and poor healing responses in the superficial digital flexor tendon and deep digital flexor tendon but not the forelimb SL.26–28

While SL microvasculature has previously been described for the forelimb of horses, to the authors’ knowledge, a similar detailed anatomic description of the microvasculature of the SL of the hind limb of horses has not been performed.26 The purpose of this study was to use contrast-enhanced CT, microvascular injection with Spalteholtz tissue-clearing technique as previously described,26 and standard histologic evaluation to describe the blood supply of the SL of the hind limb of horses. It was hypothesized that there would be a robust intraligamentous blood supply throughout the body and branches of the SL of the hind limb but a relative deficiency in intraligamentous blood supply in the proximal region (origin) of the ligament. It was also hypothesized that blood supply of the proximal region of the hind limb SL would be deficient relative to that previously described in the forelimb.26 An area of relative hypovascularity in the origin of the hind limb suspensory would offer some explanation for the distinct difference in prognosis and incidence of recurrent lameness in the hind limb as opposed to forelimb proximal suspensory disease and prompt a directed focus in future diagnostic and treatment strategies.

Methods

Eighteen hind limbs were harvested from 9 adult horses with no known history of hind limb lameness and with no known history of injury or pain associated with the hind limb SL. The horses were euthanized for reasons not associated with the study. No visible or palpable abnormalities of the distal hind limbs were noted in any subject. The horses ranged in age from 5 to 20 years (mean, 12.3 years; median, 10 years). There were 6 mares and 3 geldings. Breeds of horses used included 6 Quarter Horses, 2 Paints, and 1 Appaloosa. All limbs were transected at the middle to distal third of the tibia, then frozen and stored at −20°C. Limbs were thawed prior to injection with contrast medium or India ink.

Contrast-enhanced CT

A 14-g, 3.5-inch catheter was placed in the transected end of the cranial tibial artery at the level of the mid-distal tibia for each of both hind limbs from one horse and used to inject 150 mL of contrast medium (isovue-300; Bracco Diagnostics Inc) in each limb under steady manual pressure. After contrast injection, each limb was imaged using a 64-slice CT scanner with a 1-mm slice thickness. Following imaging, an image processing software program (OsiriX Lite; Pixmeo SARL) was used to trace the principal vasculature supplying the SL throughout its length29; 3D volume rendering was used to highlight the contrast-enhanced vascular anatomy with reference to the surrounding anatomical structures. Images taken from this software enhancement were then enhanced for clarity using an image-editing software program (Adobe Lightroom; Adobe).

Spalteholz tissue-clearing technique

A 14-g, 3.5-inch catheter was placed in the transected end of the cranial tibial artery for each hind limb. This catheter was then used to inject 120 to 150 mL of India ink under manual pressure until a minimum of 120 mL had been injected and significant backflow of ink was observed leaking from small vessels within and surrounding the transected muscle bellies of the limb. Filling of the vasculature in the distal limb was confirmed by making a small incision in the dorsal coronary band and observing leakage of India ink from the coronary band vascular plexus and the small capillaries in the dermis. One hindlimb was discarded after injection due to inadequate vascular filling in the distal limb. After injection, the limbs were refrozen at −20°C for a minimum of 72 hours before sectioning. While still frozen, limbs were then cut using a band saw in either sagittal (6) or transverse (5) orientation. Slice thickness was maintained at 4 to 7 mm for both sagittal and transverse sections. Transverse sections were labeled by attaching numbered metal tags to each section to maintain anatomic orientation and order of the samples. The tissue specimens were fixed in neutral-buffered 10% formalin after sectioning. Specimens were then cleared using the previously described modified Spalteholz technique for evaluation of microvascular anatomy of the SL.30

Histopathology

Both hind limbs from 2 horses were sectioned while frozen in transverse orientation using a band saw in approximately 4- to 7-mm thick sections and then trimmed to include only the SL and immediately surrounding bone and soft tissue structures. These tissue specimens were fixed in neutral-buffered 10% formalin, and representative samples from each hind
The limb between the second and fourth metatarsal bones, the plantar metatarsal vessels remain in close association with the SL. In the area where the SL branches, the dorsal metatarsal artery passes from the dorsolateral aspect of the limb, between the third and fourth metatarsal bones, to lie on the plantar aspect of the limb. The medial and lateral plantar and medial and lateral plantar metatarsal vessels then blend with the dorsal metatarsal artery as it branches into the medial and lateral digital vessels at the level of the distal deep plantar arch. At this level, the dorsal common digital vein also shifts from a dorsomedial orientation to a more plantar orientation; the medial and lateral plantar and medial and lateral plantar metatarsal vessels blend with the dorsal common digital vein as they do with the dorsal metatarsal artery at the level of the distal deep plantar arch. This again creates a particularly dense network of primary vasculature closely associated with the branches of the SL to provide blood supply via small branches from the parent vessels.

**Results**

**Contrast-enhanced CT**

Three-dimensional volume-rendered images shown (Figure 1) depict the principal vasculature supplying the hind limb SL. It is principally supplied by branches of the medial and lateral plantar metatarsal arteries and, to a lesser extent, the medial and lateral plantar arteries. A particularly robust principal blood supply exists in the proximal region of the SL at its origin, where the perforating tarsal vessels join with the medial and lateral plantar vessels as they form the deep plantar arch that surrounds the ligament at this level. The deep plantar arch gives rise to 2 sets of paired arteries and veins: those that travel more superficially as the continued medial and lateral plantar vessels and those that are located dorsal and/or abaxial to the SL, the medial and lateral plantar metatarsal vessels. As the SL descends the limb between the second and fourth metatarsal bones, the plantar metatarsal vessels remain in close association with the SL. In the area where the SL branches, the dorsal metatarsal artery passes from the dorsolateral aspect of the limb, between the third and fourth metatarsal bones, to lie on the plantar aspect of the limb. The medial and lateral plantar and medial and lateral plantar metatarsal vessels then blend with the dorsal metatarsal artery as it branches into the medial and lateral digital vessels at the level of the distal deep plantar arch. At this level, the dorsal common digital vein also shifts from a dorsomedial orientation to a more plantar orientation; the medial and lateral plantar and medial and lateral plantar metatarsal vessels blend with the dorsal common digital vein as they do with the dorsal metatarsal artery at the level of the distal deep plantar arch. This again creates a particularly dense network of primary vasculature closely associated with the branches of the SL to provide blood supply via small branches from the parent vessels.

**Spalteholz tissue-clearing technique**

Results from this study are similar to those described by Williams et al.28 for the forelimb SL. From its origin through the insertion of the SL branches on the proximal sesamoid bones, the SL is surrounded by a network of connective tissue containing the primary vasculature as described above and smaller branches extending from these primary vessels. This connective tissue is evident on both sagittal (Figure 2) and transverse (Figure 3) sections. The vascular branches can be observed entering directly into the SL tissues at many points throughout its length and providing a relatively uniform and plentiful microvascular supply within the ligament throughout all 3 regions. The primary vessels that contribute most substantially to vascular branches within this connective tissue network supplying the SL are the deep plantar arch and plantar metatarsal vessels in the proximal region, the plantar metatarsal vessels in the midbody region, and the dorsal metatarsal artery, distal deep plantar arch, and digital vessels in the distal region, with minor contributions from the plantar vessels in all 3 regions. In the proximal region (origin), branches of the plantar metatarsal vessels can be observed entering the dorsal aspect of the SL and coursing into the bundles of muscle and fat tissue, particularly on the transverse sections (Figure 3). Small branches from the medial plantar metatarsal artery enter the body of the SL opposite where it courses through the nutrient foramen into the third metatarsal bone as previously described in the forelimb. Throughout all 3 regions, there is a plentiful intriligamentous microvascular supply evident on sagittal (Figure 2) and transverse sections (Figure 3). Minor variations in the density of microvasculature were noted between individual horses. Specifically, for one horse, the vasculature was diffusely more prominent and plentiful throughout all regions and in both limbs in comparison to other horses. For another horse, the vasculature was slightly less prominent in comparison to other horses. In both cases, however, these variations were...
subtle and were not appreciated between regions of the ligament in the individual animals. The intraligamentous microvascular supply is particularly concentrated within the bundles of muscle and fat in the proximal region of the ligament and is more diffusely distributed throughout the ligament tissues in the body and branches (Figure 3). There do not appear to be any regions of relative avascularity in the ligament in any zone throughout its length.

**Histologic evaluation**

Histologic evaluation was performed for tissues from both hind limbs of 2 horses, sectioned from the proximal (origin), midbody (body), and distal (branch) regions of the SL (Figure 4). The findings supported those observed in samples from corresponding levels evaluated with the Spalteholz tissue-clearing technique and are similar to those described by Williams et al.\(^7\) for the forelimb SL. Abundant, diffusely distributed small vessels were observed in cross-sections within connective tissue distributed between organized bundles of collagenous tissue throughout the SL in all 3 regions. Cross-sections of small arteries and veins were particularly numerous within the bundles of muscle/adipose tissue normally present within the proximal (origin) and
midbody (body) regions of the ligament. Abundant small arteries and veins, as well as the larger principal vessels from which they originated, were evident in a network of connective tissue surrounding the SL in all 3 regions that provides the blood supply observed within the ligament. A branch of the medial plantar metatarsal artery, at the same level where a branch of this artery enters the nutrient foramen of the third metatarsal bone, gives rise to vasculature within the periligamentous connective tissue that supplies the ligament as well.

**Discussion**

This study demonstrates that the hind limb SL has a robust intraligamentous vascular supply that does not vary substantially between the origin, body, and branch regions. Intraligamentous microvasculature is abundant, particularly in comparison to the adjacent flexor tendons. The findings from this study therefore reject the authors’ hypotheses that there would be a relative deficiency in intraligamentous blood supply in the proximal region (origin) of the ligament in comparison to the other regions and in comparison to the proximal region (origin) in the forelimb SL. However, these results correlate well with the previous study describing the microvascular supply to the SL of the forelimb. Much like previous studies investigating the microvasculature of the forelimb SL and superficial and deep digital flexor tendons, this study indicates that the intraligamentous network of microvasculature is positioned between bundles of collagen fibers throughout the length of the ligament and that it originates from smaller branches of a periligamentous system of vasculature making up the principal blood supply.

The findings from this study describing the principal vasculature supplying the SL correlate well with previous descriptions outlining the location and course of the primary vessels in the tarsal and

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**Figure 4**—Representative histologic sections from the proximal (origin; A and B), midbody (body; C), and distal (branch; D) regions of equine hind limbs. A—Connective tissue containing cross-sections of small arteries and veins (arrows) is evident between bundles of ligamentous tissue. H&E stain; scale bar, 50 µm. B—Multiple cross-sections of small arteries and veins (arrows) are visible within a network of connective and adipose tissue surrounding the SL. H&E stain; scale bar, 100 µm. C—The nutrient foramen of the MTIII is continuous with the periligamentous connective tissue network. H&E stain; scale bar, 200 µm. D—The connective tissue network enters the SL and provides an extensive intraligamentous blood supply (arrows). H&E stain; scale bar, 100 µm. CTP = Connective tissue plexus. NF = Nutrient foramen.
metatarsal regions of the pelvic limb and their proximity to the SL, specifically the deep plantar arch, medial and lateral plantar metatarsal and medial and lateral plantar vessels, dorsal metatarsal artery, medial and lateral digital vessels, and distal deep plantar arch.\textsuperscript{31–35} The results from histologic samples in the present study also correlate well to other published descriptions in terms of the presence of cross-sections of arteries and veins within the bundles of muscle and adipose tissue, most significantly in the proximal region (origin).\textsuperscript{34,35} Though additional histologic descriptions of vessels observed between bundles of collagen throughout other regions of the ligament are lacking in the literature.

Previous studies have described the appearance of the origin and body of the hind limb SL on MRI and histology; these studies described the characteristic bundles of muscle and fat positioned within the SL, which are particularly prominent in the proximal one-third (origin) of the ligament.\textsuperscript{34,35} As described by the authors in this study, the intraligamentous microvasculature is found between collagen bundles of the ligament throughout its length, including in these bundles of muscle and fat tissue. In the proximal region (origin) of the ligament, the microvasculature appears to be heavily concentrated in these muscle and fat tissue bundles rather than dispersed more evenly between collagen bundles throughout the remaining cross-section of the ligament, whereas in the body and branches, where bundles of muscle and fat are less prominent or absent, the microvasculature appears to be more evenly dispersed between collagen bundles across the ligament. This results in a lack of difference in microvascular supply between the 3 regions of the ligament but a relative difference in distribution across the cross-section of the ligament between the proximal region (origin) and the midbody (body) and distal regions (branches).

The abundant intra- and periligamentous vascular supply suggests that a focal region of insufficient vasculature in normal SLs is not responsible for the commonality of or high propensity of recurrence of proximal suspensory disease in the hind limbs of horses. This is in contrast to the microvasculature of the deep digital flexor tendon, where a focal hypovascular region correlated with an area of common injury with a poor healing capacity\textsuperscript{28} but correlates well with findings for the forelimb SL microanatomy where no such hypovascular region was noted.\textsuperscript{26,28} This study provides a much-needed description of the microvascular anatomy of the equine hind limb SL that will be a valuable resource for additional research evaluating its response to injury and potential treatment strategies.

There is a significant body of work across human and veterinary-oriented literature supporting the concept that tissues with relatively low vascular supply have an increased propensity for injury and/or an inferior capacity for healing over tissues with abundant vascularity.\textsuperscript{25,36–42} Select ligaments have also shown a more limited vascular response following injury, and it has been proposed that this limited response directly relates to insufficient healing.\textsuperscript{42,43} While the results reported here do not support regionally deficient vasculature as an explanation for the commonality of injury to any one region of the SL, it does provide a basis for the study of the vascular response of this tissue to injury, which may correspond to the high likelihood of reinjury and chronic, recurrent lameness with proximal suspensory disease.

Neovascularization is an important component in the healing response of the body to injuries of tendons and ligaments, including in areas of relative avascularity.\textsuperscript{44–46} Generally, the initial response for ligaments following injury involves angiogenesis and the promotion of a disorganized but relatively hypervascular environment, followed by more uniform organization of vessels, and finally less vascular but more organized tissue.\textsuperscript{47} There is evidence to suggest that neovascularization promoting early healing may have some detrimental effects. Neovascularization in damaged tendon tissue may in fact lead to the weakening of normal tissue structure and promote a higher risk of reinjury or rupture.\textsuperscript{48}

Ultrasonography has long been considered a standard tool for evaluating the equine SL, although MRI has been shown to better match histologic changes.\textsuperscript{35,49,50} Power doppler, an ultrasonographic method that allows improved visualization of small and low-velocity blood vessels, has been shown to be a potential tool for identifying injuries in SL branches in horses.\textsuperscript{50} Because this modality highlights changes in small vessels, and because neovascularization is well established as a component of the proliferative stage of healing in tendon and ligament injury, description of the microvasculature of the SL of the hind limb of horses may help provide better interpretation of changes identified with power doppler and allow better classification and management of injuries.

The high incidence of chronic, recurrent lameness in horses with proximal suspensory pain has been attributed in part to histologic evidence of nerve compression caused by swelling of the SL within the compartment created by fascia between the second and fourth metatarsal bones plantar to the proximal SL.\textsuperscript{51} This association supports surgical transection of the deep branch of the lateral plantar nerve inner vating the proximal hind limb SL and transection of the associated fascia as a treatment for chronic lameness in these horses.\textsuperscript{22,51} Based on the present study, the principal vascular supply to the proximal region (origin) of the SL comes from vasculature within the periligamentous connective tissues; therefore, it is possible that this vasculature is affected by compression in a manner similar to the nerve. If principal vascular supply to the suspensory origin undergoes degenerative change associated with compression, this could provide some explanation as to the high rate of injury to and lameness associated with this region as well as the commonality of recurrent lameness. Further studies investigating histologic change to the principal vasculature in this region seen with clinically lame horses are warranted.

Regional differences in strain throughout the SL under tensile load conditions might also influence
differences in propensity for injury and likelihood of injury recurrence. Increased strain at common impact sites has been previously reported for patellar and supraspinatus tendons in humans and in the equine forelimb SL, with a possible correlation to increases in reinjury rates at these sites.\textsuperscript{12–15} While the increased strain for the equine forelimb SL was noted in the distal region and not the proximal,\textsuperscript{52} it is possible that a similar investigation evaluating strain patterns in equine hind limbs would yield different results.

There are some potential limitations for this study. One limitation is that, while this work provides a detailed description of microvascular anatomy of the SL, it does not account for the dynamics of blood flow and tissue perfusion in live horses. Another limitation is the possibility for incomplete vascular filling that is inherent to the nature of any cadaver-oriented vascular study. This could occur both in the contrast-injected CT samples and in the Spalteholz tissue-clearing samples and would potentially lead to a falsely low estimation of vascular anatomy. While it is certainly possible that insufficient vascular filling, as well as damage to or shrinking of vessels during the tissue fixation process, leads to underestimation of vascular supply, the results were consistent between regions in each horse, and no substantial differences were noted between horses. Contrast-enhanced MRI or CT performed in live horses may be of benefit in future studies. Finally, results in the present study were evaluated on a qualitative, but not quantitative, basis. The use of confocal laser-scanning microscopy has been described as a means of quantitative assessment of vasculature after use of the Spalteholz tissue-clearing technique.\textsuperscript{56} This technology could be applied to future equine microvascular studies.

The results of this study did not identify a region of relatively deficient microvasculature in the proximal aspect of the SL that would provide some explanation as to the high rate of injury and recurrent lameness associated with proximal SL disease in horses. However, the study contributes a much-needed description of the microvascular anatomy of this structure and provides a basis for future studies evaluating vascular response to injury and potential effects of neovascularization on tissue strength and/or chronic pain. Additional studies evaluating variations in regional strain of the hind limb SL and its vascular response to injury in horses with acute proximal suspensory pain are warranted.

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