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B-mode and power Doppler ultrasonography of the equine suspensory ligament branches: A descriptive study on 13 horses

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Abstract

Ultrasonography is routinely used to achieve the diagnosis of equine suspensory ligament desmopathy. In human medicine, power Doppler ultrasonography has also been found to be useful for the diagnosis of tendon/ligament injuries. The aim of this prospective, pilot study was to assess the presence or absence of power Doppler signal in suspensory ligament branches and compare B-mode findings with power Doppler findings in suspensory ligament branches of lame and non-lame limbs. Thirteen horses were used (eight lame horses, with lameness related to pain in the suspensory ligament branches, and five non-lame horses). Ten lame limbs and 24 sound limbs were assessed by B-mode and power Doppler ultrasonography. The severity of power Doppler signal was scored by two independent readers. The B-mode ultrasonographic examination revealed abnormalities in branches of lame limbs and in branches of sound limbs. Suspensory ligament branches that were considered normal in B-mode showed no power Doppler signal. However, power Doppler signal was detected in suspensory ligament branches that were abnormal in B-mode, both in lame and sound limbs. Power Doppler scores were subjectively higher in suspensory ligament branches of lame limbs and in branches with more severe B-mode changes. Findings supported the use of power Doppler as an adjunctive diagnostic test for lame horses with suspected suspensory desmopathy.

KEYWORDS

desmopathies, horse, ultrasound, vascular

1 | INTRODUCTION

Suspensory desmopathies are a common cause of lameness in sport and pleasure horses.[1,2] The injuries of the suspensory ligament can be divided in relation to the affected area: lesions restricted to the proximal one third (proximal suspensory desmopathies), lesions of the middle third, sometimes extending into the proximal third (body lesions) and lesions of the medial and/or lateral branch (branch lesions).[2] Pain originating from the origin of the suspensory ligament has been diagnosed in horses with increasing frequency in recent years[3–6] and lesions of the suspensory ligament branches have been regularly reported.[1,6–8] Brightness-mode (B-mode) ultrasonography is an easily available modality to image the suspensory ligament and its bone surface insertions. B-mode ultrasonography is considered essential for an accurate diagnosis of suspensory ligament desmopathies[2] and several studies reported the use of B-mode ultrasonography to evaluate the suspensory ligament.[1,3,9]

In human medicine, ultrasonography with color or power Doppler was also found to be useful for the assessment of tendon/ligament injuries. The Doppler technique detects and monitors increased blood flow in musculoskeletal disease[10,11] which is interpreted as a sign of pathology in patients with clinical signs of tendinopathy.[12] Power Doppler ultrasonography, in comparison to color Doppler, has better sensitivity to detect flow from small vessels and low velocity flow at microvascular level.[13] Power Doppler ultrasonography does not measure velocity or direction but is very sensitive to flow.[14] It is therefore almost angle independent and is not subject to aliasing.[15] Furthermore, the relative independence of beam to vessel angle produces a better delineation of tortuous vessels.[13] The value of power Doppler ultrasonography in depicting low-velocity blood flow at the microvascular level in different types of human tissues has been proven[16] and the highly significant correlation between power Doppler ultrasonography and histopathological findings supports the value of this imaging technique.[16] In human non-inflammatory tendon disease (so called tendinosis), Doppler activity is also detected in painful patients and it is thought to demonstrate neovascularization of the affected structures.[17]

Previous studies have suggested that Doppler activity may have the same pattern in humans and in horses.[18,19] However, only few reports mention the use of Doppler in the diagnosis of equine chronic tendon injuries and use Color Doppler[18–20] and there are no publications on the use of power Doppler ultrasonography for the assessment of increased blood flow in the musculoskeletal system and in particular in the suspensory ligament. Because of the easy applicability of the technique, a combination of B-mode and power Doppler ultrasonography could be used in horses as a noninvasive and easily available complementary examination to standard clinical assessment for evaluating suspensory ligament desmopathy both in daily management and clinical trials as used in human medicine.[21] Because horses having sustained suspensory ligament injuries in the past may have persistent ultrasonographic hypoechoic B-mode abnormalities[22] or hyperechoic changes as result of chronic fibrosis, the use of Doppler (and especially power Doppler because of its higher sensitivity)[13] may potentially help to distinguish old from active suspensory ligament lesions.

This study aimed to describe presence or absence and signal strength of power Doppler signal in suspensory ligament branches of horses with or without lameness. We hypothesized that suspensory ligament branches with normal B-mode appearance in sound limbs would not show power Doppler signal, while power Doppler signal would be present in suspensory ligament branches that were considered abnormal in B-mode in lame limbs, and that power Doppler signal score would be higher in suspensory ligament branches with more severe abnormalities at B-mode examination.

2 | METHODS

2.1 | Horses and clinical assessment

The study was a prospective, pilot design. The protocol was approved by an institutional animal care committee. Two groups of horses were used: a group of horses presented at the equine clinic for lameness investigation and undergoing ultrasonography as a part of lameness work up (group L) and a group of sound horses (group S) owned by the Veterinary Faculty. With informed client consent, eight client-owned horses with a lameness considered related to pain of one or both suspensory ligament branches based on the clinical examination and/or local analgesia were included prospectively during a 6-month period. Inclusion criteria for horses in group L were a final clinical diagnosis of suspensory ligament branch desmopathy with lameness considered related to the desmopathy based on the clinical examination, with or without diagnostic analgesia. The severity of lameness at trot was graded from 1 to 5 on a scale of 5. The S group included five horses owned by the Veterinary School and were examined ultrasonographically as a part of student training in musculoskeletal ultrasonography, under approval of the ethical committee of the institution. Inclusion criteria for group S were absence of lameness at the time of the ultrasonographic study and no known history of lameness related to a suspensory ligament desmopathy. Sample sizes for the two groups were based on clinical caseload and availability of horses owned by the Veterinary school. All decisions for subject inclusion or exclusion were made by the first and last author in consensus.

All horses underwent a complete clinical examination by one board-certified orthopedic surgeon to assess presence or absence of lameness. For lame horses, the lame limb, the severity and duration of lameness, and the result of flexion tests and local anesthesia were recorded.

2.2 | Ultrasonography protocol

All ultrasound examinations were performed by the last author. The observer was aware of the group status of the horse at the time of examination. Ultrasonography was performed at rest, meaning that on the day of the ultrasonographic examination, exercise was limited to the clinical examination at trot in hand and a minimum time of 45 min had passed between the clinical and the ultrasonographic examinations. The suspensory ligaments of both the lame limb and the contralateral limb were examined ultrasonographically as a part of clinical work-up for horses of group L. Horses of group S were examined ultrasonographically as a part of last-year students teaching.

Suspensory ligament branches were evaluated ultrasonographically using two nonportable ultrasonographic machines (Aloka Prosound SSD-3500, Mitaka, Tokyo, Japan and MyLab 70, Esaote, Genova, Italy) with a linear 7–13 MHz transducer. All B-mode ultrasonographic examinations were performed on the weight-bearing limb by the same experienced operator. The limbs were prepared by fine clipping, washing, and application of ultrasonographic gel. The ultrasonographic images of the suspensory ligament branches were obtained with a stand-off pad. The suspensory ligament branches were imaged from the bifurcation to the insertion in transverse and longitudinal plane.

All the branches assessed by B-mode ultrasonography were examined using power Doppler ultrasonography. The power Doppler examination was performed at rest by the same operator (same operator of B-mode ultrasonographic examination). The power Doppler ultrasonography was performed using the same transducer as B-mode examination, with the limb bearing no weight in a flexed position and without using the stand-off pad. Very little pressure was applied on the transducer. Power Doppler images were obtained in the transverse plane.

The sensitivity of the ultrasonographic machines was optimized for low flow with the lowest possible pulse repetition frequency and the lowest possible wall filter. The Color gain was set just below the noise level. The focus was placed where the highest sensitivity was required or just below the region of interest. The color box was large and placed on the entire section area of the branch examined.

2.3 | Image analysis

Images obtained in B-mode and power Doppler ultrasonography (group L and group S) were retrospectively reviewed by one experienced reader (S.R.) to record ultrasonographic features and location of B-mode abnormalities and presence of power Doppler signal. The reader was aware of horse group status at the time of image analysis.

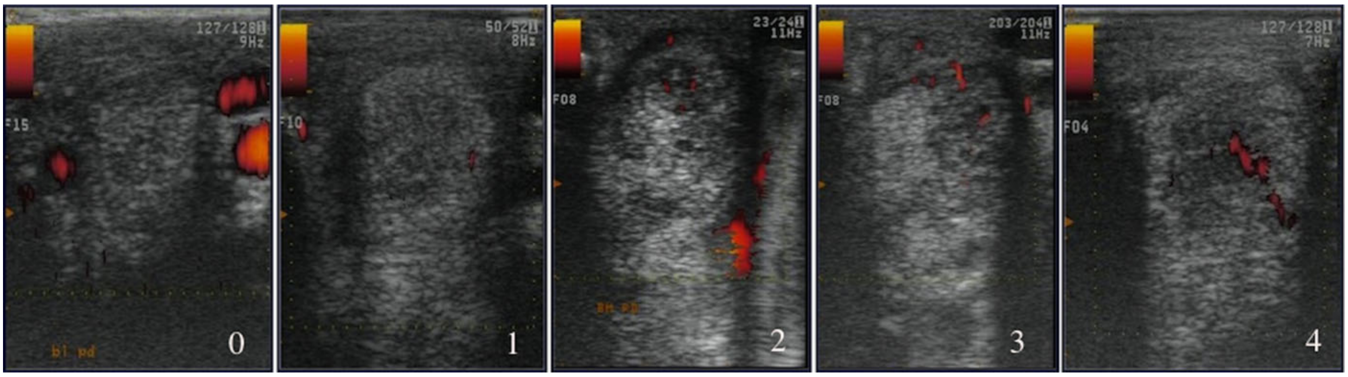

FIGURE 1 Transverse power Doppler ultrasonographic images of the suspensory ligament branches illustrating criteria used for scoring the power Doppler ultrasonographic signal. From left to right: 0, no power Doppler signal; 1, single spot of power Doppler signal; 2, more than one spot of power Doppler signal; 3, more than one spot of power Doppler signal and a single vessel signal; and 4, more than one spot of power Doppler signal and two vessel signals [Color figure can be viewed at wileyonlinelibrary.com]

The order of image interpretation was not randomized. B-mode abnormalities were subjectively classified by the same reader as mild, moderate, or severe. Lesions were defined as mild when echogenicity changes were homogeneous and unifocal, there was a change in shape but no major change in cross-section area in comparison to the contralateral limb; moderate when echogenicity changes were multifocal, and there was a change in shape with or without a change in cross-section area; and severe when echogenicity was heterogeneous, architecture was modified, and there was a change in shape and cross-section area.

For all branches, the same reader selected the ultrasonographic image with maximal power Doppler activity together with the corresponding B-mode ultrasonographic image (if no power Doppler activity was detected one image per branch was selected at the same level of the B-mode abnormalities). The selected power Doppler ultrasonographic images were then graded blindly by two independent readers (final year large animal resident and board certified veterinary radiologist) for intra-ligamentous power Doppler signal using a semi-quantitative scale from 0 to 4 (0, absence no power Doppler signal; 1, a single spot of power Doppler signal; 2, more than one spot of power Doppler signal; 3, more than one spot of power Doppler signal and a single vessel signal; 4, more than one spot of power Doppler signal and two vessel signals confluent or not confluent) (Figure 1).

Statistical analyses were selected and performed by V.B., using an online clinical research calculator (Vassarstats.net) software. Agreement of the two readers for power Doppler scoring was calculated using a Cohen's Kappa test.

3 | RESULTS

The group of horses included nine geldings and four mares, aged 4–22 years (mean 14 years), mainly Warmbloods (seven). Horses were used for general purpose (eight), dressage (two), 3-day eventing (one), and racing (two).

3.1 | Clinical features in horses

In the L group, three horses had unilateral forelimb lameness, three horses had unilateral hindlimb lameness, and two had bilateral hindlimb lameness. Severity of lameness at trot varied from 1 to 4 on a scale of 5 (median value 2.5). In three cases the lameness was recent (lameness of a duration less than 1 month) and in five cases the lameness was old (recurrent or consistent lameness of a duration of more than 3 months). For seven of the eight horses, the suspicion of desmopathy of one or more branches was present at physical examination. Five horses showed a palpable thickening of the affected branch and pain was induced by direct pressure applied to the injured branch in the three cases with recent lameness onset. Passive flexion of the fetlock exacerbated lameness in two horses with chronic lameness. Perineural and/or intra-articular analgesia were performed in five horses. Lameness persisted after analgesia of the palmar/plantar digital nerves at the level of mid pastern and in four cases lameness was abolished by a "low 4-point block" (palmar/plantar nerves and palmar/plantar metacarpal/tarsal nerve). In one horse perineural analgesia of the palmar/plantar metacarpal/metatarsal nerves was not performed and lameness was considered significantly reduced after intra-articular analgesia of the metacarpo-phalangeal joint. The three horses with recent lameness showing suspensory ligament branch swelling and pain had no diagnostic analgesia performed. In two patients fetlock degenerative joint disease was suspected to contribute to the lameness together with suspensory ligament branch desmopathy.

All horses of group S were free of lameness the day of the ultrasonographic examination.

3.2 | B-mode ultrasonographic findings

Ten lame limbs and six controlateral sound limbs were examined in horses of the L group (six unilateral lameness and two bilateral lameness) and 18 limbs were examined in the S group for a total of 68 suspensory ligament branches examined (20 on lame limbs and 48 on sound limbs).

The B-mode ultrasonographic examination revealed abnormalities in 43 branches, 18 on lame limbs, and 25 on sound limbs. The abnormal ultrasonographic features detected at B-mode were heterogeneous subcutaneous peri-ligamentous thickening, enlargement, change in shape, poor margin definition, heterogeneous architecture and echogenicity, hypoechoic areas, irregular bony surface of proximal sesamoid bones (in five limbs of which two showed an avulsion fracture). B-mode abnormalities were present in 18 of 20 (90%) suspensory ligament branches of lame limbs and 25 of 48 (52%) suspensory ligament branches of sound limbs were abnormal at B-mode. In sound limbs, B-mode abnormalities were classified as mild in 23 of 25 (92%) and as moderate in two of 25 (8%) limbs. In lame limbs, B-mode abnormalities were classified as severe in seven of 18 (38.8%) limbs, moderate in four of 18 (22.2%), and mild in seven of 18 (38.8%) limbs.

3.3 | Power Doppler ultrasonographic findings

None of the branches that were normal at B-mode ultrasonography showed power Doppler signal (Figure 2A and B). Power Doppler signal was detected in 23 of 43 (53.5%) abnormal branches at B-mode, both in lame (11) and sound limbs (12) (Figure 3A and B). Power Doppler signal was located in the area of the branch abnormal at B-mode. Agreement between the two independent blind readers was good for presence/absence of power Doppler signal (Kappa = 0.786) and good between power Doppler scores assigned (unweighted Kappa = 0.6771, weighted Kappa = 0.749). Power Doppler scores were different between lame and non-lame limbs (Figure 4), with lame limbs (in particular acutely lame) having scores ranging from 1 to 4 (Figure 5A,B) in comparison to sound limbs having scores ranging from 0 to 1 (Figure 6A and B) (except two branches in which the score of 2 was given by one of the two readers). Suspensory branches with lesions classified as severe at B-mode had the highest power Doppler scores

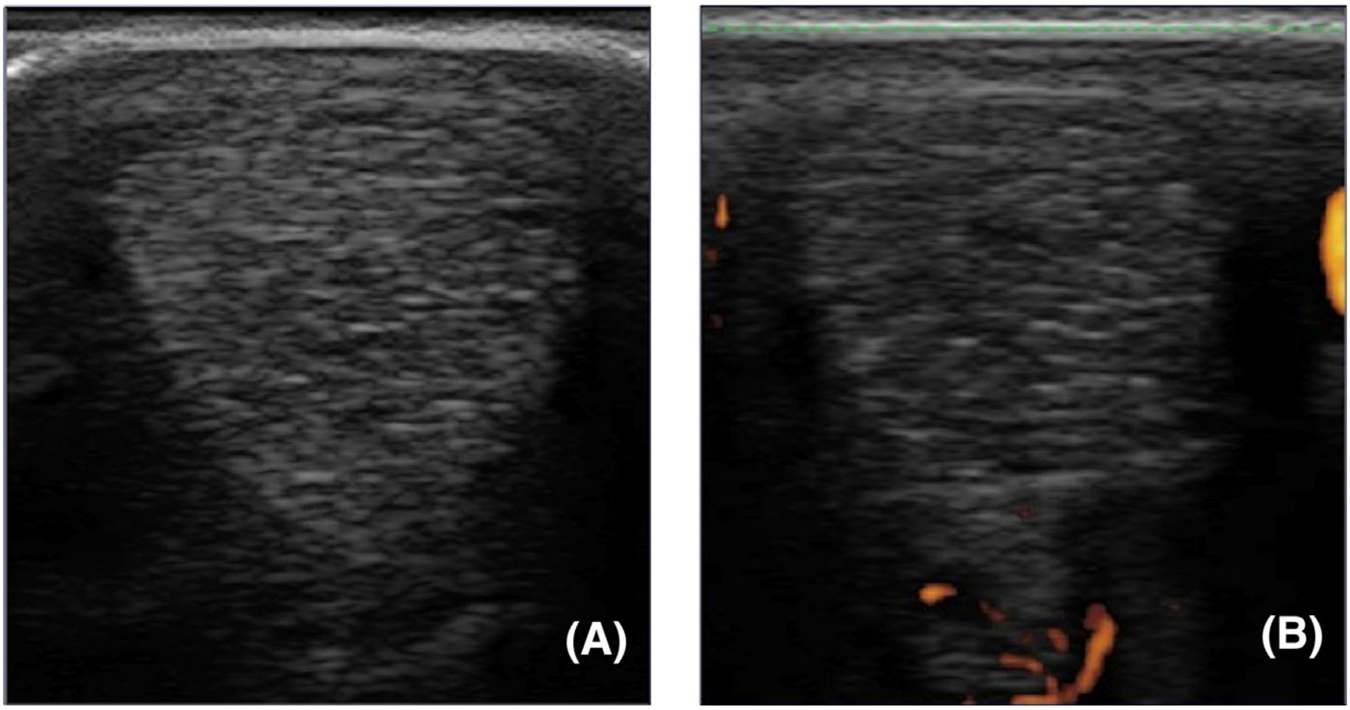

FIGURE 2 Transverse ultrasonographic images (A, B-mode and B, power Doppler) of a medial suspensory ligament branch in a forelimb of a horse free of lameness. A, The suspensory ligament branch is normal in B-mode. B, Power Doppler image obtained at the same level: no Power Doppler signal is visible within the suspensory ligament branch [Color figure can be viewed at wileyonlinelibrary.com]

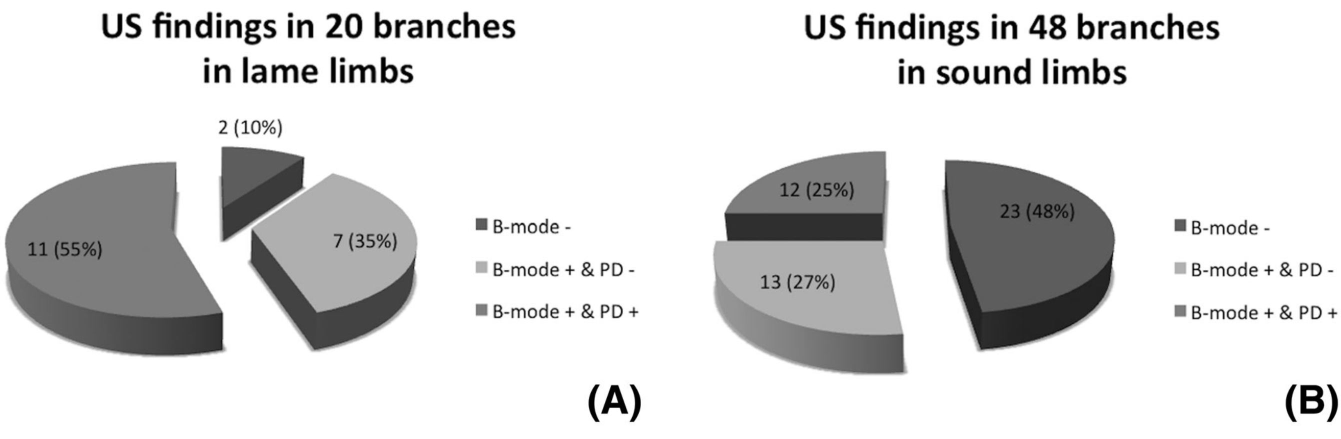

FIGURE 3 A, Graph showing the ultrasonographic findings in 20 suspensory ligament branches in lame limbs. B, Graph showing the ultrasonographic findings in 48 suspensory ligament branches in sound limbs. US, ultrasonographic; PD, power Doppler; +, present; −, absent

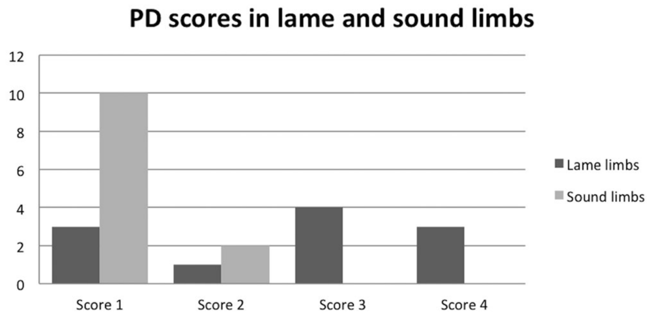

FIGURE 4 Graph showing Power Doppler scores in lame and sound limbs. PD, power Doppler

(Figure 7). The acutely lame limbs (three horses) had scores ranging from 3 to 4 in comparison to chronically lame limbs (five horses) having scores ranging from 1 to 4.

4 | DISCUSSION

In agreement with our hypothesis, no power Doppler signal was present in suspensory ligament branches with normal B-mode appearance while power Doppler signal was seen in abnormal branches in B-mode.

However, power Doppler signal was present not only in lame limbs that were abnormal in B-mode but also in some sound limbs that were abnormal in B-mode. Scores were subjectively higher in lame limbs with more severe B-mode changes.

B-mode ultrasonography is commonly used to investigate equine tendons and has been demonstrated to be a reliable and cost-effective method to locate abnormalities of suspensory ligament branches.[1,2,7,8,23–25] At B-mode ultrasonographic examination, lesions of the suspensory ligament branches are reported to improve slowly, frequently persist long-term (longer than 18 months),[2] and the degree of ultrasonographic abnormality does not appear proportionate to the degree of lameness.[6] Persistence of ultrasonographic abnormalities beyond the return to clinical normality and athletic activity is common.[1] Relative old suspensory ligament lesions or mild suspensory ligament lesions, no longer inducing lameness, may therefore appear abnormal at B-mode ultrasonographic examination.[1,7,22] Moreover, in an ultrasonographic study of the forelimb suspensory ligament branches of 60 Thoroughbred racehorses in training, B-mode ultrasonographic abnormalities appeared to exist in a small but significant proportion of animals without history or clinical indication of injury.[7] In the present study, B-mode abnormalities were seen in both groups of horses thus supporting the importance of recognizing that the absence of lameness does not preclude visible abnormality at B-mode ultrasonography on a suspensory ligament branch.[8] Higher prevalence of B-mode abnormalities in group L in lame limbs is obviously due to case selection as this was based on desmopathy of the suspensory ligament branch as a cause of lameness used as main inclusion criterion for horses in group L.

In humans, power Doppler ultrasonography has been extensively used to assess increased blood flow of tendons and ligaments.[10,12,17,26,27] The finding of increased blood flow at power Doppler ultrasonography in chronically painful tendinopathy has

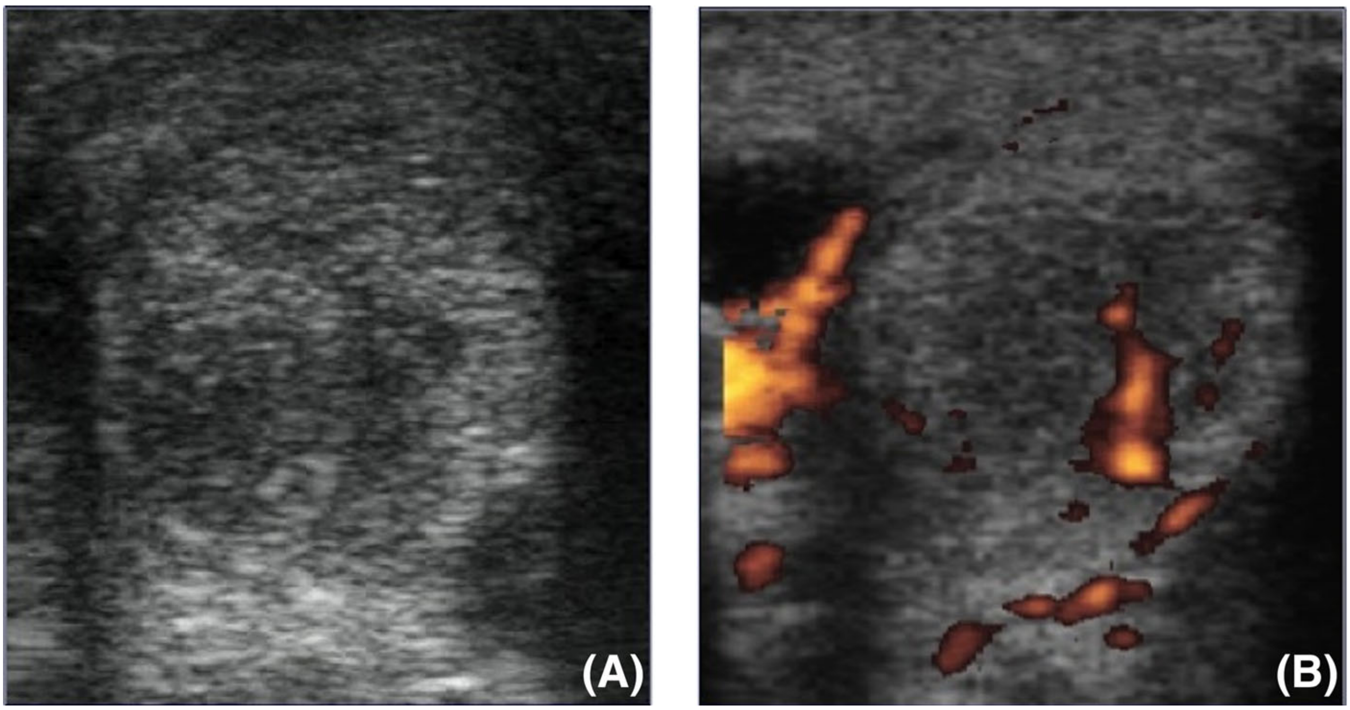

FIGURE 5 Transverse ultrasonographic images (A, B-mode; B, power Doppler) of a medial suspensory ligament branch in a hindlimb of a horse with acute lameness. A, Severe B-mode abnormalities: A heterogeneous/hypoechoic, ill-defined area is visible mainly in the central part of the branch associated with increased volume and change in shape (rounded appearance). B, Power Doppler image obtained at the same level showing power Doppler signal (score 4) [Color figure can be viewed at wileyonlinelibrary.com]

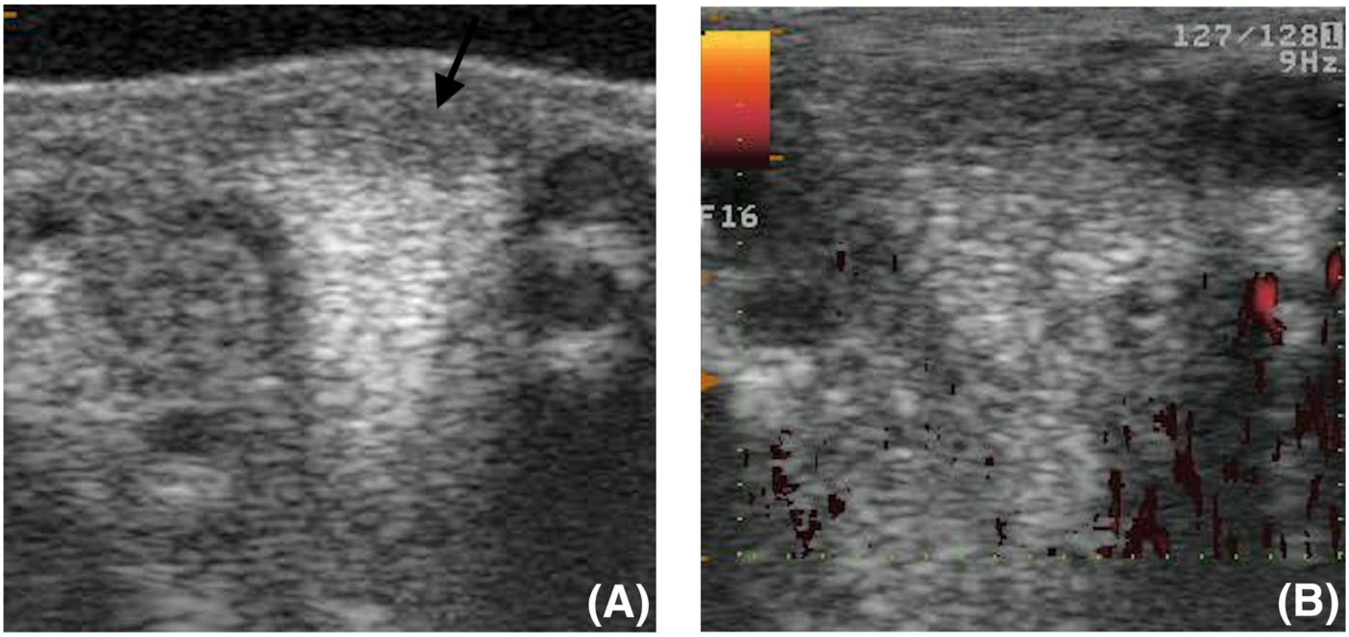

FIGURE 6 Transverse ultrasonographic images (A, B-mode and B, Power Doppler) of a lateral suspensory ligament branch in a hindlimb of a horse free of lameness. A, Mild B-mode abnormalities: A hypoechoic area is visible in the superficial part of the branch (black arrow). B, Power Doppler image obtained at the same level: No Power Doppler signal is visible [Color figure can be viewed at wileyonlinelibrary.com]

been correlated to pain, since these vessels are normally not seen in pain-free tendons and neoinnervation accompanies neovessel formation.[17,28] In the present study power Doppler activity was observed in 23 of 43 suspensory ligament branches (53.5%) that showed abnormalities at B-mode ultrasonographic examination. Kristoffersen and collaborators[19] have previously described neovascularization detectable at Color Doppler ultrasonography in areas with structural changes in chronically affected equine suspensory ligaments, as demonstrated in chronically painful human tendons.[21] The absence of power Doppler signal in some of the abnormal branches at B-mode ultrasonographic examination is in contrast with the results of previous studies on color Doppler where all horses with chronic tendon injuries demonstrated color Doppler signal.[18,19] This difference may be explained by the fact that increased blood flow is a dynamic finding, more pronounced during acute exacerbation of pain but

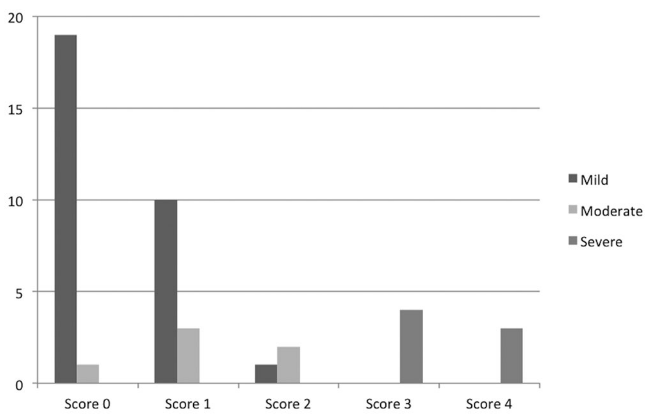

FIGURE 7 Graph comparing the severity of B-mode abnormalities with power Doppler scores

disappearing at inconstant rates.[29] The inconstant presence of power Doppler signal in abnormal branches at B-mode in the present study is also in accordance with reports in humans showing that tendons with tendinopathy may or may not show evidence of Doppler signal.[11,29] A different degree of stretching or relaxing a tendon during power Doppler ultrasonographic examination may also have influenced the ability to detect power Doppler signal.[30] However, as the limb was held in a non-weight-bearing position to avoid tension on the branches and for optimal visualization of potential blood flow in the present study and in the studies of Kristoffersen and collaborators[19] and Boesen and collaborators[18], major differences in tendon tension are unlikely.

Normal branches at B-mode showed no power Doppler signal and this is in accordance with the two previous reports in horses and with most publications in human medicine.[17–19] However, minimal or mild power Doppler signal was seen in horses with abnormalities in B-mode in non-lame limbs of lame horses and in asymptomatic horses. This is in contrast with most studies in human tendinopathy which suggest that, in asymptomatic patients, tendons are supplied by a vasculature that is so thin that it is undetectable with current power Doppler equipment.[27,29] However, some authors have found power Doppler signal in tendons of healthy subjects with no B-mode changes and suggest questionable relevance of single microvessels in asymptomatic tendons considering the increased sensitivity of modern power Doppler equipment.[31] In our study, sound limbs showing power Doppler signal all had B-mode abnormalities. This suggests some degree of previous structural damage and it is therefore unlikely that the finding of minimal or mild power Doppler signal represents a normal feature detected because of higher sensitivity of power Doppler equipment. The hypothesis of the presence of minimal to mild power Doppler signal as being a sign of mild old tendinopathy that is clinically irrelevant in relationship to the level of work the horse on the day of the clinical examination seems more likely in the present study.

In humans, power Doppler signal has been demonstrated to occur in both acutely and chronically affected tendons and is referred as neovascularization. This neovascularization is believed to be the result of different mechanisms depending of the age of the lesion (vasodilation in acute damage and neo-angiogenesis in chronic disease)[21,27,29] but power Doppler signal is unable to give information about the process occurring in the affected tendon. However the involvement of an inflammatory response in any process of neovascularization is suggested by some authors.[32,33] As no histopathological examination is available in the present study it is impossible to make a correlation between the exact nature and stage of the lesions and the presence and degree of power Doppler signal in the horses of the present study.

Exercise induces peripheral vasodilation and the relative increased blood flow seen after exercise and indicated by Doppler activity is considered by some authors to be a physiological response.[12] In the present study, all horses were examined at rest. On the other hand, horses were exercised before ultrasonographic examination in the study of Kristoffersen and collaborators.[19] Exercising the horses during 15 min at trot before ultrasonographic examination would have potentially increased the visibility of miscrovasculature in suspensory ligament branches that presented abnormalities in B-mode and in which no power Doppler signal was detected at rest. However, because all lame horses included in the present study were client-owned horses, exercising them for a time period long enough to produce peripheral vasodilation was considered unfeasible due to the risk of exacerbating ligament damage. Moreover, by examining all horses at rest, any physiological Doppler response that may have persisted as a consequence of suspensory ligament stress during exercise[12] has been excluded as potential bias.

Finally, although the suspensory ligament is the remnant of the third interosseous muscle and its branches have a completely tendon-like structure,[34] it is important to bear in mind that the suspensory ligament is considered a ligament as it joins two bones; the literature in human sport medicine mainly report data about tendon Doppler activity.[11,12,27] The patellar tendon in humans has, similar to the equine suspensory ligament, two bony attachment and numerous studies have demonstrated its vascular activity by power Doppler and color Doppler[10,35,36] suggesting that Doppler changes occur independently from direct continuation into a muscular belly.

Although this study has been conducted in a limited number of horses and has limitations, it demonstrated the feasibility of a routine power Doppler exam of the equine suspensory ligament branches. The results also suggest a potential correlation between intra-ligamentous increased blood flow detected at power Doppler ultrasonography and B-mode ultrasonographic abnormalities as well as between severity of power Doppler signal and lameness of the limb, with suspensory ligament branches that were normal in B-mode in non-exercised horses being power Doppler signal-free and power Doppler signal being more evident in branches with more severe B-mode changes in lame horses. However, because B-mode abnormalities and power Doppler signal were also detected in non-lame horses, further longitudinal studies monitoring power Doppler activity over time and studies comparing power Doppler activity with histopathologic findings are needed to better understand the mechanism responsible for power Doppler signal and to correlate increased blood flow with the stage of the lesion. These may lead in the future to use of power Doppler as a routine clinical complement during follow-up to establish the best time for return to work in horses where B-mode abnormalities persist.

LIST OF AUTHOR CONTRIBUTIONS

Category 1

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(b) Acquisition of Data: Rabba S, Grulke S, Verwilghen D, Evrard L, Busoni V

(c) Analysis and Interpretation of Data: Rabba S, Grulke S, Verwilghen D, Evrard L, Busoni V

Category 2

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(a) Final Approval of the Completed Article: Rabba S, Grulke S, Verwilghen D, Evrard L, Busoni V

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