TITLE: Mineralization can be an incidental ultrasonographic finding in equine tendons and ligaments

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Clinical and Imaging Features of Common Ultrasonographically Detectable Tendon and Ligament Mineralisation in Horses

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Running head: Mineralisation in Equine Tendons and Ligaments

Abstract

Although tendon/ligament mineralisation is recognised in horses, its clinical features have not been reported in detail. Our aims were therefore to identify the structures most commonly affected by ultrasonographically detectable mineralisation and, for these, determine frequency of diagnosis and clinical features including association of mineralisation with lameness and outcome.

Two case series (retrospective and prospective) were analysed and the frequency of mineralisation in lame animals estimated (observational descriptive design).

Mineralisation was reported in 27 horses (22 retrospective) - most commonly in deep digital flexor tendons (10) and suspensory ligament branches (8), representing 10% and 7% (estimated) respectively of horses with injuries to these structures. Two deep digital flexor tendon and 3 suspensory ligament branch cases showed bilateral mineralisation. Deep digital flexor tendon mineralisation was restricted to the digital flexor tendon sheath, most commonly in the proximal sheath (± sesamoidean canal), and 7/10 cases involved hindlimbs. Suspensory ligament branch mineralisation was visible in the same ultrasound window as the proximal sesamoid bones in 10/11 limbs and 6/8 cases involved forelimbs. Previous corticosteroid medication was a feature of only 1 deep digital flexor tendon and 1 suspensory ligament branch case.

Mineralisation was associated with lameness in some but not all limbs. Foci within the deep digital flexor tendon preceded hypoechoic lesion formation in 2 limbs. Of cases with deep digital flexor tendon or suspensory ligament branch injury only, 1/3 and 2/3 respectively became sound. Further investigation is necessary to understand the pathogenesis of mineralisation and so lead to specific treatments.

Introduction

Mineralisation of tendons and ligaments has been reported in both equine and human patients. In horses, mineralisation is an occasional finding during ultrasound examination and has been described within the superficial and deep digital flexor tendons, the suspensory ligament, the peroneus tertius tendon, the plantar ligament, the nuchal/supraspinous ligament and the biceps brachii tendon, and the palmar/plantar annular ligament.[1-4] It has been suggested that deep digital flexor tendon mineralisation is most prevalent in middle aged Warm Blood horses used for Dressage or Show Jumping and that previous intrathecal corticosteroid medication may be a risk factor.[5] In addition, superficial digital flexor tendon mineralisation has been associated with intratendinous injection with methylprednisolone acetate.[6] However, determining the clinical significance of tendon and ligament mineralisation is difficult at present, given a lack of data, in particular regarding the association of mineralisation with lameness and outcome.

Half of human patients with mineralisation of the rotator cuff tendons are asymptomatic,[7] but in symptomatic individuals, treatment aimed at resolving mineralisation can improve comfort.[8,9] These observations suggest that mineralisation can be a primary cause of tendon pain. In addition, in human patients, associations between pain and both mineralisation morphology and the presence of Doppler signal have been reported.[7]

The diagnosis of rotator cuff mineralisation using ultrasonography and radiology has been compared and 3 types of mineralisation described.[10] The first type was a hyperechoic focus with well-defined shadow, the second a hyperechoic focus with a faint shadow, and the third a hyperechoic focus with no shadow, although some false positive diagnoses were identified in the latter group.

Equine tendinopathy has been reported to frequently have a bilateral presentation[11] while, in human patients, mineralisation of the rotator cuff tendons was only present bilaterally in 10% of affected patients.[12] In contrast, a murine model of unilateral tendon injury resulted in accelerated mineralisation both at the site of injury and in the contralateral tendon.[13]

Our first aim was to identify the structures most commonly affected by ultrasonographically detectable mineralisation in horses. Second, we aimed to determine for these commonly affected structures (the deep digital flexor tendon and suspensory ligament branch) the frequency of diagnosis and bilateral occurrence of mineralisation. Our third aim was to report the clinical features of these cases including association of mineralisation with corticosteroid medication, lameness, Doppler signal, other evidence of injury and outcome. The study employed an observational descriptive design.

Materials and Methods

Cases of mineralisation, diagnosed by the senior author using ultrasonography, were identified from hospital records for patients examined between April 1999 and April 2013 at the [redacted] A 'case' was defined as a single animal affected by mineralisation within a single structure (for example, the deep digital flexor tendon on 1 limb) or bilaterally within the same structure. The occurrence of mineralisation in more than 1 structure within the same animal was considered as a separate case for each affected structure. Ultrasonographic examinations performed in each pair of fore or hind limbs included weight bearing transverse and longitudinal grey scale scans with a standoff. Later examinations also included transverse off incidence (Figure 1)[14] scans and examination for colour Doppler signal (non-weight bearing, without standoff). Most animals were sedated (alpha 2 agonist ± butorphanol) to enable examination. Diagnostic criteria were the presence of hyperechoic foci casting acoustic shadows within or on the surface of a tendon or ligament visible in either or both transverse and longitudinal planes. Surface hyperechoic foci casting a shadow were included because of the difficulties in establishing the deep surface of the mineralisation and their intimate association implied involvement with the structure.

Cases of enthesiopathy (categorised as echogenic shadows continuous with bone), avulsion fractures (categorised as echogenic shadows within tendon/ligament adjacent to bone with corresponding defects within the bone), mineralisation adjacent but outwith the tendon or ligament were excluded. Cases of foreign body penetration were excluded based on the absence of three features: visible or reported wounding; reverberation artefacts; and defined foreign body shape.

A second phase of the study included cases of tendon/ligament mineralisation diagnosed at the [redacted] September 2014 – November 2015 (prospective cases) using the same inclusion criteria. The ultrasonographic examination of cases in this phase was the same as for the later cases of the retrospective study. In both phases, animals underwent single or multiple examinations dependent upon clinical progress.

Clinical features of the 2 most common categories (deep digital flexor tendon and suspensory ligament branch) were evaluated. For the retrospective phase, ultrasound images, where available, were reviewed to verify the written reports. For both phases, the dimensions of the mineralisation were measured from representative images (both phases) using the Fiji distribution of ImageJ[15]. Mineralisation morphology was subjectively graded as 'well defined' or 'poorly defined' and distribution assessed as 'focal' or 'diffuse'. Information on return to work was obtained by telephone call to the owner (retrospective and prospective cases).

The frequency of mineralisation diagnosis in lame horses was estimated as follows. First, for the deep digital flexor tendons and suspensory ligament branches the number of animals with any ultrasonographic lesions (i.e. not limited to mineralisation) diagnosed by the senior author during the years 2000, 2006 and 2012 was determined from hospital records and expressed as an estimated annual mean. Then, for each structure, estimated frequency of mineralisation diagnosis (%) was calculated as 100*(Sum of mineralisation cases April 1999 – April 2013)/ [14*(estimated annual mean number of cases with any ultrasonographic lesions)].

132 Data collected from cases included: signalment when mineralisation was identified and
133 limbs involved; location of mineralisation; treatment history including
134 peritendinous/ligamentous medication; alteration of mineralisation (assessed
135 subjectively); association of mineralisation with lameness, duration of lameness prior to
136 identification of mineralisation; association with other signs of
137 tendinopathy/desmopathy; presence of colour Doppler signal (not all cases); association
138 with surgical findings; and outcome. Association with lameness was defined as
139 'associated', 'not associated' or 'unproven association', based on intrathecal analgesia
140 or history (deep digital flexor tendons), and regional analgesia or pain on palpation
141 (suspensory ligament branches).

Results

Identification of most commonly affected structures

The distribution of tendon/ligament mineralisation in 27 cases is listed in Table 1. The majority of mineralisation was detected in the deep digital flexor tendons and the suspensory ligament branches (37% and 30% of cases respectively). In no horse was mineralisation documented in more than 1 type of tendon or ligament.

For the retrospective phase, ultrasound images were available for all deep digital flexor tendon cases and all ligament branch injury cases but 1.

Equipment used (retrospective and prospective phases)

During the study, Vingmed System 5 and Vivid 7 (GE Medical Systems Limited, Chalfont St Giles, Bucks, UK) ultrasound systems with linear probes (7.5-14 MHz) were used. To examine colour Doppler signal with the Vivid 7 system a Doppler frequency of 7.5 MHz with a pulse repetition frequency of 1.0 kHz were used; the settings used with the System 5 machine were not available.

Estimated frequency of mineralisation diagnosis

For deep digital flexor tendons and suspensory ligament branches, mineralisation was estimated to be present in 10% and 7% respectively of animals with ultrasonographic abnormalities of these structures. During the 3 years used to estimate these frequencies, the mean number (± standard deviation) of deep digital flexor tendon and suspensory ligament branch injury diagnoses were 5.7 (± 0.6) and 7 (± 2.6) respectively.

Details of cases including bilateral occurrence of mineralisation

Ten cases of deep digital flexor tendon mineralisation were reported, 4 were female and the remainder were geldings. Breed type was reported as: Thoroughbred (1); Arab (1); Cleveland Bay (1); Thoroughbred cross or Warmblood (5); Cob (1) or Unknown (1). Mineralisation was found in mature horses with a median age of 13.5 years (range 3-18 years). In 7 cases, 1 or both hind limbs were affected, the remaining cases involved the forelimbs. In 2 cases mineralisation was identified bilaterally (1 forelimb and 1 hindlimb pair).

Of the 8 cases of suspensory ligament branch mineralisation, 2 were female and the remainder geldings. Breed type was reported as: Thoroughbred (2); Thoroughbred cross or Warmblood (4), Irish Sports horse (1) or Crossbreed (1). The median age was 8 years (range 5-17 years). In 6 cases the forelimbs, and in the remainder the hind limbs, were involved. Mineralisation was documented unilaterally in 4 cases, bilaterally (2 or more branches on contralateral limbs) in 3 cases and either uni- or bilaterally in the other case (record unclear).

Clinical features

Location of mineralisation

Deep digital flexor tendon mineralisation was recognised only within the digital flexor tendon sheath which was divided into 2 anatomic levels: proximal sheath (± sesamoidean canal) and distal. Mineralisation was restricted to the proximal sheath (± sesamoidean canal) in 6 cases (1 bilateral, 5 unilateral; Figure 2) and was within both the proximal (± sesamoidean canal) and distal digital flexor tendon sheath in 4 cases (1 bilateral, 3 unilateral).

In general, mineralisation was situated within the centre and palmar/plantar aspects of the deep digital flexor tendon. In no case was mineralisation restricted to the dorsal tendon surface.

In all but 1 case (unilateral) suspensory ligament branch mineralisation was visible in the same longitudinal ultrasound window as a portion of the respective proximal sesamoid bone.

Mineralisation dimensions, morphology and distribution

All deep digital flexor tendon mineralisations were poorly defined but focal within the affected anatomic levels. (Figures 1,2,4 and 6). Mineralisations in the proximal digital flexor tendon sheath (12 limbs/10 cases) measured 11.3 ± 5.9 mm long in longitudinal images and 4 ± 3.0 mm wide in the transverse images (mean ± standard deviation). Distal digital flexor tendon sheath mineralisations (5 limbs/4 cases) measured 13.8 ± 9.4 mm long in the longitudinal images and 2 ± 0 mm wide in the transverse images.

Suspensory ligament branch mineralisation was also poorly defined but diffusely distributed and difficult to accurately measure in the majority of cases (Figure 3). In longitudinal images, the length of the branch over which the mineralisation was distributed varied between a few millimetres to at least 30 mm.

Association between mineralisation and corticosteroid medication

204 For the deep digital flexor tendon cases, there was a history of intrathecal medication
205 prior to documentation of mineralisation in only 1 case (unilateral). In this horse the
206 digital flexor tendon sheath of the affected deep digital flexor tendon had been
207 medicated 6 weeks prior to referral.

208 For the suspensory ligament branch cases, in 1 case (unilateral) the fetlock joint had
209 been medicated within 2 months of suspensory ligament branch mineralisation being
210 identified on the contralateral limb. In a second case, bilateral forelimb fetlock joint
211 medication had been performed 2 years prior to the identification of unilateral forelimb
212 suspensory ligament branch mineralisation. There was no history of joint medication
213 recorded for any other cases.

214 <u>Alteration of mineralisation</u>

215 Three cases of deep digital flexor tendon and 2 cases of suspensory ligament branch
216 mineralisation had sequential examinations. For 2 cases of deep digital flexor tendon
217 mineralisation (each unilateral) no progression was noted between examinations 1-3
218 months apart and in the third case mineralisation was documented in the non-lame leg
219 initially but detected in the lame leg 1 month later. Mineralisation was noted to become
220 more focal for 1 suspensory ligament branch case (unilateral) after 7 weeks (Figure 3)
221 and unchanged over 10 months in a second case (bilateral).

222 <u>Association of mineralisation with lameness</u>

223 Mineralisation of the deep digital flexor tendon was associated with lameness based on
224 a positive response to intrathecal analgesia in 6/12 limbs (6 cases). Of this subgroup,
225 mineralisation was within the proximal digital flexor tendon sheath (± sesamoidean

canal) in 2 and for the other 4 the mineralisation was present in both the proximal and distal digital sheath. In 5 of these limbs, additional deep digital flexor tendon lesions were identified either ultrasonographically (in 2 cases after a 5-11 week delay) and/or tenoscopically, and in 1 limb a superficial digital flexor tendon lesion (not mineralisation), considered more likely to be the cause of the lameness, was present.

However, mineralisation was not associated with lameness in 3/12 limbs (3 cases). One case was sound (1 limb; mineralisation in the proximal digital flexor tendon sheath). In another case mineralisation was bilateral but lameness completely eliminated by unilateral intrathecal analgesia (mineralisation in the proximal digital flexor tendon sheath on both limbs). In a third case (1 limb; mineralisation in the proximal digital flexor tendon sheath) lameness was acute onset (manica flexoria tear) in the non-mineralised limb. For the remaining 3/12 limbs (2 cases) an association with lameness was unproven. In 1 case, unilateral lameness of the mineralised limb was markedly improved but not resolved following a palmar digital block. In the second case with bilateral mineralisation, lameness was substantially improved but not eliminated by unilateral analgesia. As the lameness was not completely eliminated in these 2 cases, an association between mineralisation and lameness cannot be excluded.

Suspensory ligament branch mineralisation was associated with lameness by either regional analgesia or palpation in 6/11 limbs (6 cases). But, similar to the deep digital flexor tendons mineralisation, not all mineralisation in the suspensory ligament branch was associated with lameness (2/11 limbs). One case was sound when examined (unilateral) and in 1 case with bilateral mineralisation, blocking the contralateral limb eliminated the lameness and no switch occurred. For the remaining 3/11 limbs (3 cases) the association of mineralisation with lameness was unproven due to incomplete information or failure to eliminate lameness on the contralateral limb.

Duration of lameness prior to identification of mineralisation

Of the 6 cases with lameness localised to the mineralised deep digital flexor tendon, the duration of lameness before documentation of mineralisation (by the referring clinician or at the [redacted]) was available for 4 cases (all unilateral) and ranged from 2 days to 6 months.

For the 6 cases with lameness localised to the mineralised suspensory ligament branch, information on duration of lameness before documentation of mineralisation ranged from 7 weeks to 5 months for 3 cases (1 uni- and 2 bilateral).

Association with other signs of tendinopathy/desmopathy

Deep digital flexor tendon mineralisation was associated with other ultrasonographic evidence of tendinopathy, as defined by the presence of hypoechoic lesions and/or adjacent poor fibre pattern in 6 limbs (5 cases). For 3/6 limbs (3 cases) there was other evidence of tendinopathy at the same time as the mineralisation was identified and these lesions were associated with lameness in 2 limbs (positive intrathecal analgesia) and not associated in the third limb. In 2/6 limbs (2 cases; retrospective phase) mineralisation was the only abnormality detectable ultrasonographically at the first examination but between 5 and 11 weeks later these cases developed hypoechoic lesions within their deep digital flexor tendons (Figure 4). In both of these cases lameness was localised to the digital flexor tendon sheath by intrathecal analgesia at the first examination. In 1/6 limbs (retrospective phase), mineralisation was identified 4 weeks after evidence of tendinopathy and there was a positive response to intrathecal analgesia at that time. There was no other ultrasonographic evidence of tendinopathy in the remaining 6 limbs (5 cases). These cases were either sound (1 unilateral), lame in the contralateral limb (3 limbs), or had other lesions (2 limbs; intrathecal superficial digital flexor tendon lesion and foot pain respectively) within the lame limb, which were considered more likely to explain the lameness.

Eight limbs (6 cases) with suspensory branch ligament mineralisation showed other evidence of desmitis, including entheseopathy, heterogenous fibre pattern/hypoechoic foci (at least 5 limbs), and enlargement. These lesions were associated with lameness in 5 limbs (5 cases), not associated in 1 limb and had an unproven association in 2 limbs (2 cases). Two limbs (2 cases) showed no other evidence of desmitis. Of these cases, 1 was sound and the second underwent surgery to treat impingement on the suspensory ligament by the second metacarpal bone. In the remaining limb, mineralisation was documented following surgery to remove a proximal sesamoid bone fracture and details of the pre-operative examination were not available.

Mineralisation and Doppler signal

Results for 6 deep digital flexor tendon cases (6 limbs) evaluated for colour Doppler signal are shown in Figure 5. In both limbs where Doppler signal was present in the tendon, the mineralisation was associated with lameness (positive intrathecal analgesia). There were 2 limbs positive to intrathecal analgesia but without deep digital flexor tendon Doppler signal, and 1 of these had an intrathecal superficial digital flexor tendon lesion (with Doppler signal) thought to be the cause of the lameness.

293 An example of Doppler signal related to deep digital flexor tendon mineralisation and
294 adjacent hypoechoic areas (2 limbs/cases) is shown in Figure 6. Hypoechoic areas
295 were distinguished from blood vessels by appearance, location and absence of Doppler
296 signal. In both limbs, the hypoechoic areas were identified at a second ultrasound
297 examination, but in neither case was Doppler signal tested for when the horse was first
298 scanned.

299 Three of the suspensory branch ligament cases (3 limbs) were examined for colour
300 Doppler signal. Like the deep digital flexor tendon cases, the single limb with signal
301 adjacent to the mineralisation had lameness localised to the affected branch. The other
302 2 limbs demonstrated no Doppler signal related to the mineralisation: 1 case diagnosed
303 with second metacarpal bone impingement on suspensory ligament (Doppler signal
304 related to the impingement only); and the second case was sound when examined.

305 <u>Association with tenoscopic abnormalities</u>

306 Tenoscopy was performed on 6 limbs (5 cases) affected by deep digital flexor tendon
307 mineralisation. In 5 of these limbs there was no defect on the surface of the tendon. In 1
308 limb the epitenon overlying an intratendinous deep digital flexor tendon defect was
309 disrupted and in another a longitudinal deep digital flexor tendon tear was found.

310 <u>Outcome</u>

311 Follow-up was available for 8 cases of deep digital flexor tendon mineralisation: 3 cases
312 where deep digital flexor tendinopathy was the sole diagnosis and 5 cases where there
313 was an additional injury.

Of the cases where deep digital flexor tendinopathy was the sole diagnosis, 1 case was competing at medium level dressage 5 years after treatment by palmar annular ligament resection and controlled exercise (unilateral mineralisation within the proximal and distal digital flexor tendon sheath). The second case (bilateral mineralisation in the proximal digital flexor tendon sheath) remained lame 14 months after diagnosis and the third was euthanased due to persistent lameness (unilateral mineralisation in the proximal digital flexor tendon sheath). Of the 5 cases with additional injuries, 1 case was returned to eventing after surgical treatment of a manica flexoria tear in the non-mineralisation affected limb (unilateral mineralisation within the proximal digital flexor tendon sheath). Four cases were either lame at final examination (>/= 8 months after initial) or remained lame according to the owner.

Follow-up was available on 3 cases in which suspensory branch ligament mineralisation was detected. One case remained sound 3 months after examination (unilateral), and 1 raced 5 times following treatment (unilateral). One case remained lame 10 months after diagnosis (bilateral). Suspensory branch ligament desmitis was the sole diagnosis in these cases.

Discussion

The deep digital flexor tendon and suspensory ligament branches were the structures most commonly affected by mineralisation and an estimated 10% and 7% of cases of deep digital flexor tendon and suspensory ligament injury respectively demonstrate this feature. Deep digital flexor tendon mineralisation was restricted to the digital flexor tendon sheath which is the typical location for deep digital flexor tendinopathies outwith the foot.[5,16] However, mineralisation may present in this tendon without other evidence of active tendinopathy or evidence of lameness in that limb, which is consistent with the observation in human patients that not all rotator cuff tendon mineralisation is associated with pain.[7] Nevertheless mineralisation can precede the development of hypoechoic foci in lame animals.

Mineralisation can also occur bilaterally and was found in 20% of deep digital flexor tendon and 43% of suspensory ligament branch cases. Although Webbon[11] documented a higher rate of bilateral injury occurrence - 67% of superficial digital flexor tendons examined grossly - mineralisation is only 1 of many gross features of tendinopathy.[17] The bilateral occurrence of tendinopathy, including mineralisation, could be explained by the common loading history of affected tendons or a compensatory increase in loading in the contralateral structure following unilateral injury. There is also evidence that central nervous system signalling may be involved in the bilaterality of tendon disease.[18] Rotator cuff tendon mineralisation has been reported bilaterally in only 10% of human patients, which might relate to species differences in the prevalence of bilateral pathology, or differences between studies in sensitivity to detect bilateral changes.

In vivo experimental evidence supports the suggestions that previous intrathecal or intratendinous corticosteroid injection may promote tendon mineralisation.[19] However in the current series, previous medication on the affected limb was reported in only 1 case of deep digital flexor tendon and 1 case of suspensory ligament branch mineralisation and none had been treated intra-tendinously/ligamentously. Not all medication may have been reported. Nevertheless, our records suggest that corticosteroid medication was unlikely to have been a predisposing factor in most cases.

It is interesting that mineralisation was found much less frequently in the superficial digital flexor tendon compared with the deep digital flexor tendon, despite the former being the most commonly injured flexor tendon.[20] Differences in tendon matrix composition[21] may be a contributory factor. Tendons and ligaments are known to develop a cartilage phenotype in response to compressive load[22-24] and this phenotype is observed at the level of the fetlock joint where the deep digital flexor tendon changes direction and is compressed against the proximal scutum, offering the possibility that mineralisation forms here by endochondral ossification and could be an extreme response to compressive loading. An alternative explanation is that poorer vascularisation, known to be present where tendons wrap around bony prominences,[25] contributes to mineralisation within this tendon. Why the suspensory ligament branches are a predilection site for mineralisation is less obvious but similar mechanisms may apply.

The molecular events within the tendon/ligament matrix leading to mineralisation are not well understood. In human patients, tendon and ligament mineralisation may involve ossification (endochondral or intramembranous) or deposition of calcium salts by other mechanisms.[26] Within the rotator cuff, the most common site of tendon mineralisation in human patients,[27] the process has been described as 'incomplete endochondral ossification'.[28] Rodents predictably respond to tendon injury by true endochondral ossification.[26,29] The nature of mineralisation within the equine tendons and ligaments has not been clearly established and may differ between deep digital flexor tendons and suspensory ligament branches. A recent report described mineralised foci excised from the palmar/plantar annular ligaments of ponies as either osseous metaplasia or dystrophic mineralisation.[4] A mineralised focus with a deep digital flexor tendons dissected by the senior author had a granular appearance less consistent with bone. These observations suggest that, like humans, mineralisation within equine tendons may vary in composition, perhaps dependent upon location.

Histopathological examination is required for definitive diagnosis of tendon/ligament mineralisation but is impractical in most clinical cases. Therefore, the lesions reported in this study are presumed mineralisation. In 1 study, the authors included hyperechoic foci which did not cast acoustic shadows in their criteria for ultrasonographic diagnosis of human rotator cuff tendon mineralisation which resulted in some false positives, when compared with radiography.[10] To minimise this risk we included only cases with acoustic shadowing which may have reduced our sensitivity. It is also possible that some under-reporting occurred in the retrieval of data during the retrospective phase of the study. However, it is unlikely that these limitations will have altered the pattern of clinical features which we observed.

Fibre damage was present at the time of (3 cases/4 limbs) or after (2 cases/limbs in retrospective phase) identification of mineralisation in the deep digital flexor tendon cases. For at least 1 of the latter cases, our records indicate that the original images were reviewed at the time the hypoechoic lesion was first identified and confirmed that, ultrasonographically, mineralisation may precede fibre disruption. Signs of fibre disruption were present in at least 5 limbs with suspensory ligament branch mineralisation. Three hypotheses may explain the association between mineralisation and lameness and tendon/ligament fibre disruption. In healing rabbit ligaments, an association between the presence of flaws and a reduction in their material properties has been documented.[30] In the same way, mineralised foci may promote fibre failure and in turn pain. Secondly, mineralisation may promote an inflammatory response in the adjacent tendon causing pain and fibre weakening. A vigorous inflammatory response adjacent to mineralisation has been identified in human supraspinatus tendons.[31] Thirdly, it is feasible that mineralisation does not directly contribute directly to tendon pain/fibre rupture but is an incidental change (a ‘bystander’). However, the improvement in patient comfort following surgical decompression of mineralised deposits in rotator cuff tendons suggests that mineralisation can be an active contributor to tendon pain.

The ability to discriminate significant mineralisation from incidental findings would be of great clinical value. In cases with existing lameness it may be possible to treat before the development of hypoechoic lesions. Further, in non-lame animals it may be possible to identify those likely develop lameness in the future, which would be useful in the context of a pre-purchase examination. One study reported the presence of Doppler signal within the mineralised area in 21/57 symptomatic human patients but in none of

422 the asymptomatic cases (P<0.005).[7] These authors also identified that larger and
423 fragmented mineralisations were significantly associated with pain.

424 Doppler signal was associated with mineralisation in 2/3 limbs where the lameness was
425 thought to arise from the mineralised deep digital flexor tendon and in a single case
426 where the lameness associated with suspensory branch ligament desmitis. Doppler
427 signal was absent where either the horse was sound or there were other causes of
428 lameness identified (2 suspensory ligament branch and 3 deep digital flexor tendon
429 cases). Unfortunately, there was insufficient information available to conclude if Doppler
430 signal could be used to predict the occurrence of lameness or development of
431 hypoechoic lesions.

432 One of the 3 horses with deep digital flexor tendinopathy as the sole diagnosis returned
433 to work. This finding was not unexpected as a guarded prognosis been reported for this
434 condition previously (excluding cases with mineralisation), with 7/24 cases returning to
435 intended use.[32] A guarded prognosis has also been reported for suspensory ligament
436 branch desmitis, with 10/23 cases returning to intended use.[3]

437 Surprisingly, this case of deep digital flexor tendon mineralisation with a positive
438 outcome had the most proximodistally extensive distribution pattern (within the proximal
439 and distal digital flexor tendon sheath). However, mineralisation may vary significantly in
440 its dorsopalmar/plantar thickness, which is difficult to assess ultrasonographically due to
441 the anatomic constraints of this area. In a future prospective study, serial radiography
442 could help assess mineralisation size, distribution and progression; although it may be a
443 challenge to monitor mineralisations within the sesamoidean canal. Computed

tomography may be the ideal technique in this respect, but is presently impractical in clinical studies.[13] Magnetic resonance imaging may also help determine which mineralisations are contributing to lameness.[33]

Typically human patients with rotator cuff tendon mineralisation show clinical and radiographic/ultrasonographic resolution of signs with conservative treatment such as nonsteroidal anti-inflammatories, corticosteroid medication and physical therapy.[34] The marked improvement in the appearance of the mineralisation in 1 suspensory ligament branch case is therefore unsurprising although there was insufficient follow up to say if this occurred frequently in horses. Related to this, mineralisation became detectable between examinations 1 month apart in 1 deep digital flexor tendon case. This observation suggests that it may be erroneous to assume that ultrasonographic evidence of mineralisation reflects a tendon/ligament injury of many months duration. Indeed, osteophytes, which like some presentations of tendon mineralisation form by ossification,[35] can become radiographically apparent in a little as 2 weeks.[36] Within the human population, when rotator cuff mineralisation fails to resolve with minimal treatment, extracorporeal shock wave therapy, needle decompression and arthroscopic removal may be successful.[10] Needling is probably only warranted when the deposits are focal and liquid or granular rather than ossified. With greater understanding of the mechanisms of equine tendon/ligament mineralisation, these treatments may be considered appropriate.

In agreement with a previous suggestion, most cases in this series were middle aged and larger breed types.[5] In human patients, rotator cuff tendon mineralisation and Achilles tendon mineralisation are reported to occur more frequently in females and

467 males respectively.[26] A slightly higher proportion of cases with either deep digital flexor
468 tendon and suspensory ligament branch mineralisation were geldings rather than mares
469 in this series. This distribution, and the frequency of mineralisation which we report, may
470 relate to differences in the activities of mares and geldings attending our hospital and
471 should be generalised to the wider population with caution.

472 In humans, there is some limited evidence associating endocrine disorders with rotator
473 cuff tendon mineralisation.[37] Pituitary pars intermedia dysfunction and metabolic
474 syndrome are the most obvious candidates for a possible similar link in equine
475 tendon/ligament mineralisation, being the most common endocrinopathies affecting
476 horses.[38] However, investigating such a link is beyond the scope of our data.

477 Other systemic disorders associated with tendon or ligament mineralisation in human
478 patients include ankylosing spondylitis and the rare conditions, fibrodysplasia ossificans
479 progressiva and progressive osseous heteroplasia which involve generalised soft tissue
480 mineralisation.[26] These disorders do not appear relevant to our patients. An association
481 between dietary imbalance and tendon/ligament mineralisation has not been reported in
482 any species.

483 No cases of deep digital flexor tendon mineralisation within the hoof capsule were
484 reported in this study, despite injury to this structure being a common cause of foot pain.
485 [16]. Deep digital flexor tendon mineralisation within the foot is detectable
486 ultrasonographically.[39] However, transcuneal ultrasonography was performed less
487 frequently by the senior author during the study compared with pastern and
488 metacarpal/metatarsal scans. Further, this approach is likely much less sensitive than

489 radiography or magnetic resonance imaging to detect deep digital flexor tendon
490 mineralisation, given the limited size of the transcuneal window.

491 In conclusion, this report confirms that mineralisation can be associated with lameness,
492 but may also be an incidental finding. Doppler imaging may offer additional support for
493 the significance of mineralisation, but more but more data are required to confirm a
494 pattern. A further pathological study is also recommended to understand the nature of
495 mineralisation and better and determine if specific measures (e.g. shockwave and
496 needling) are rational as treatment strategies.

497

498

List of Author Contributions

Category 1

a) Conception and Design

[redacted]

b) Acquisition of Data

[redacted]

c) Analysis and Interpretation of Data

[redacted]

Category 2

a) Drafting the Article

[redacted]

b) Revising the Article for Intellectual Content

[redacted]

Category 3

a) Final Approval of the Completed Article

[redacted]

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Competing Interests

None declared.

Ethical considerations

Oversight was provided by the [redacted] Review Board (Project number URN 2015 1364)

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639 **Table 1**. Distribution of Mineralisation within Equine Tendons and Ligaments.

Structure	Number of cases
Deep digital flexor tendon	10 (2)
Suspensory ligament branch	8 (1)
Superficial digital flexor tendon	2
Intersesamoidean ligament	2 (1)
Oblique distal sesamoidean ligament	2 (1)
Lateral collateral ligament of stifle	1
Manica flexoria	1
Palmar carpal ligaments	1
Total number of cases	27

640 (Number of cases examined prospectively)

641

642

List of Figure Legends

Figure 1. Examples of the use of on- (left) and off- (right) incidence transverse views to aid identification of mineralisation (arrowheads). This mineralisation is poorly defined but focal.

Figure 2. Transverse and longitudinal ultrasound images from deep digital flexor tendon case 3 (A, B) and deep digital flexor tendon case 4 (C,D) showing mineralisation (arrowheads) in the proximal and distal digital flexor tendon sheath. In both locations the mineralisation is poorly defined but focal.

Figure 3. Example of mineralisation of the suspensory ligament branch when first identified (A) and 7 weeks later (B) by which time it has become more focal but remains poorly defined (arrowheads).

Figure 4 Transverse ultrasound images from deep digital flexor tendon case 5 showing poorly defined focal mineralisation at first examination (A; arrowhead). There was no other ultrasonographic evidence of tendinopathy at this time. One month later a hypoechoic lesion (arrow) had developed adjacent to this mineralisation (B).

Figure 5. Overview of 6 limbs with deep digital flexor tendon (DDFT) mineralisation (M) evaluated for colour Doppler signal. + = positive Doppler signal in the affected tendon; - = absence of Doppler signal in the affected tendon; (number of limbs). Association of mineralisation with lameness was based on positive digital flexor tendon sheath analgesia. *SDFT = intrathecal superficial digital flexor tendon lesion (not mineralisation) which demonstrated Doppler signal.

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Figure 6. Transverse (A) and longitudinal (B) ultrasound images from deep digital flexor tendon case 7 showing Doppler signal associated with poorly defined focal mineralisation (arrowheads). This mineralisation was associated with lameness based on a positive response to intrathecal analgesia.

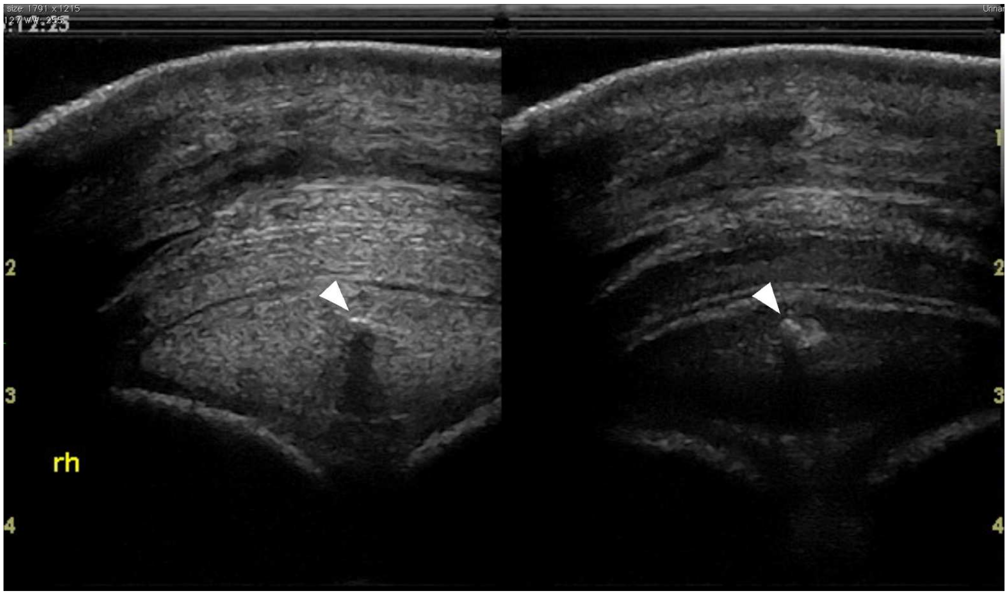

Figure 1. Examples of the use of on- (left) and off- (right) incidence transverse views to aid identification of mineralisation (arrowheads). This mineralisation is poorly defined but focal.

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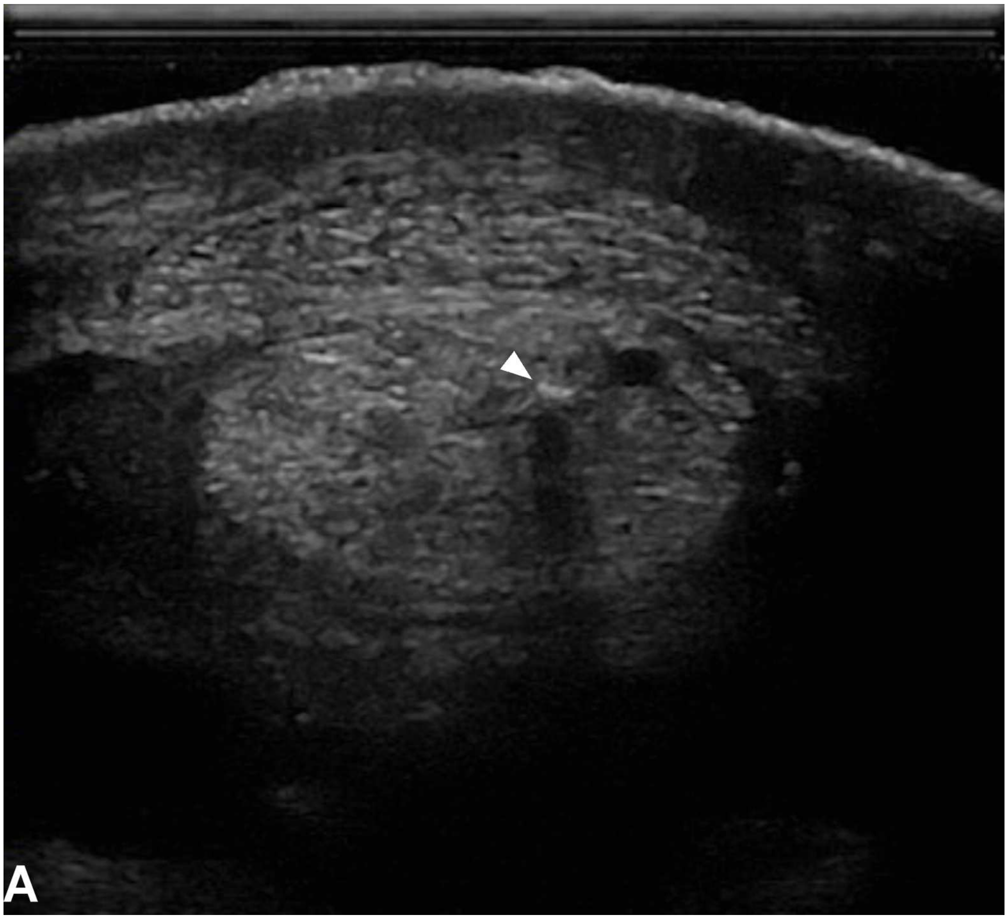

Figure 2. Transverse and longitudinal ultrasound images from deep digital flexor tendon case 3 (A, B) and deep digital flexor tendon case 4 (C,D) showing mineralisation (arrowheads) in the proximal and distal digital flexor tendon sheath. In both locations the mineralisation is poorly defined but focal.

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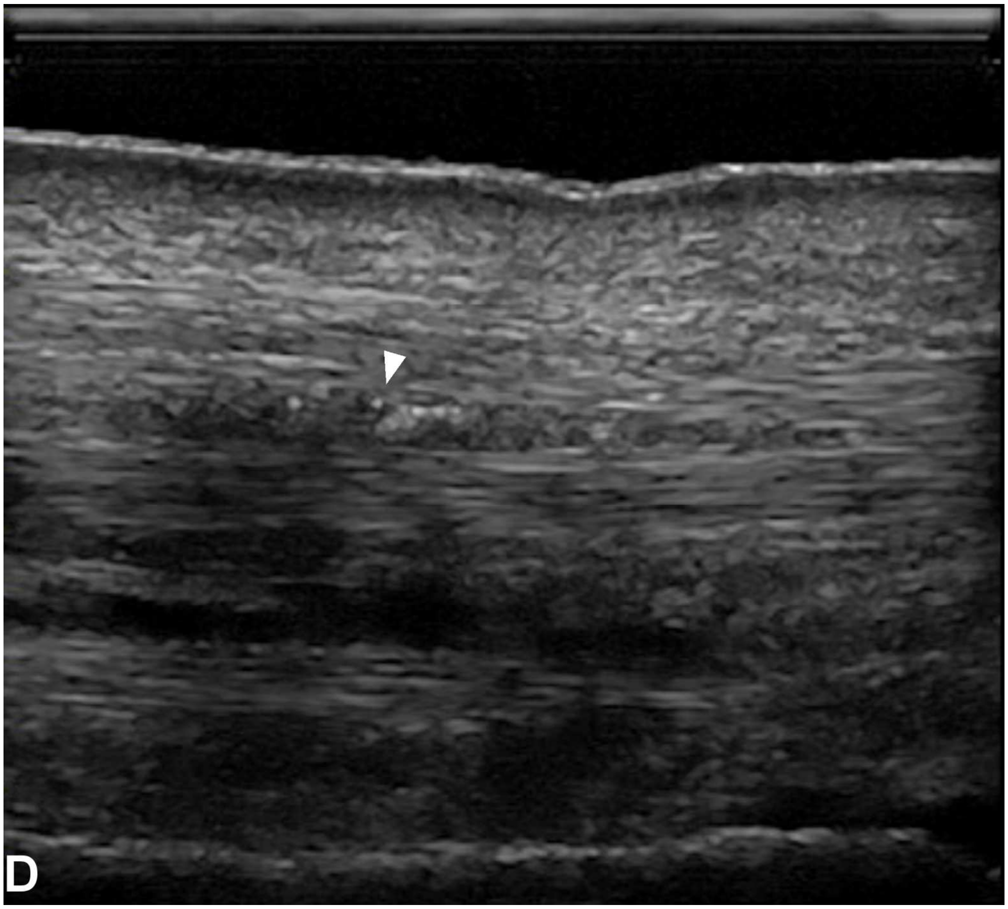
169x174mm (300 x 300 DPI)

169x173mm (300 x 300 DPI)



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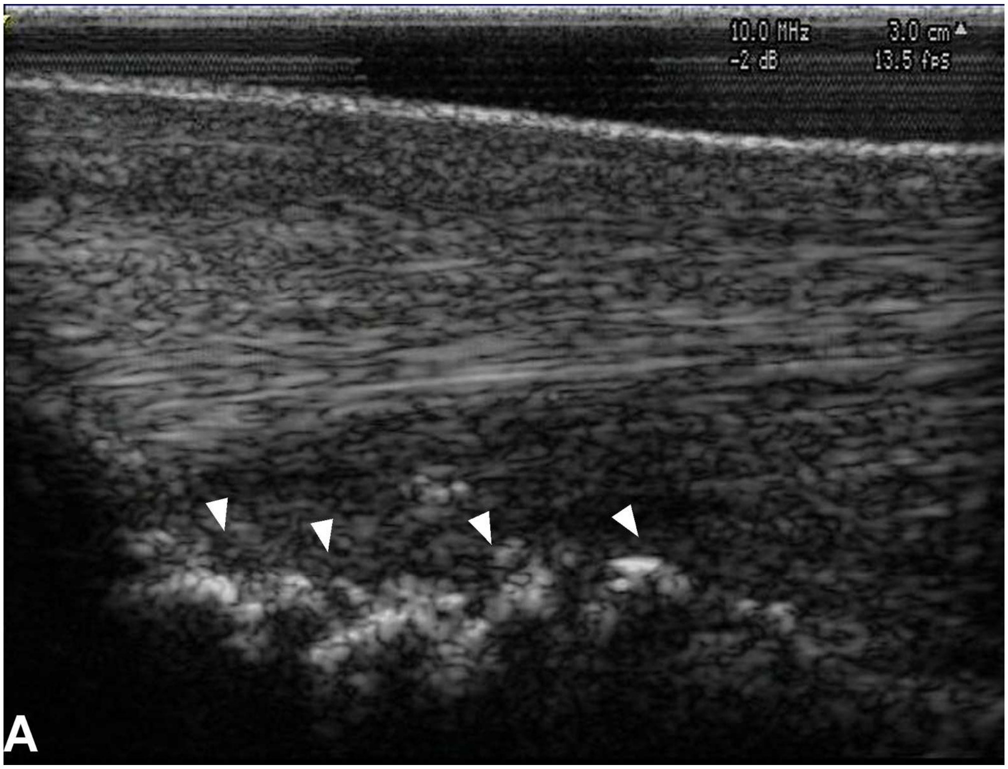

Figure 3. Example of mineralisation of the suspensory ligament branch when first identified (A) and 7 weeks later (B) by which time it has become more focal but remains poorly defined (arrowheads).

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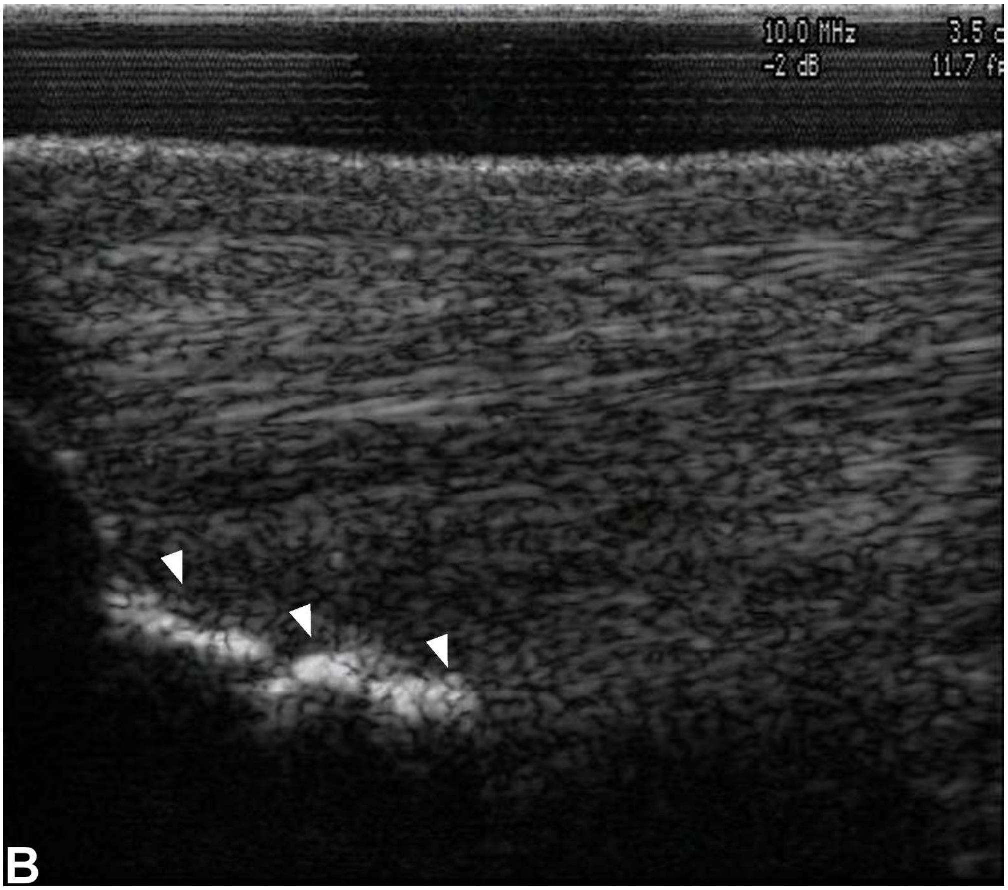

169x149mm (300 x 300 DPI)

Figure 4 Transverse ultrasound images from deep digital flexor tendon case 5 showing poorly defined focal mineralisation at first examination (A; arrowhead). There was no other ultrasonographic evidence of tendinopathy at this time. One month later a hypoechoic lesion (arrow) had developed adjacent to this mineralisation (B).

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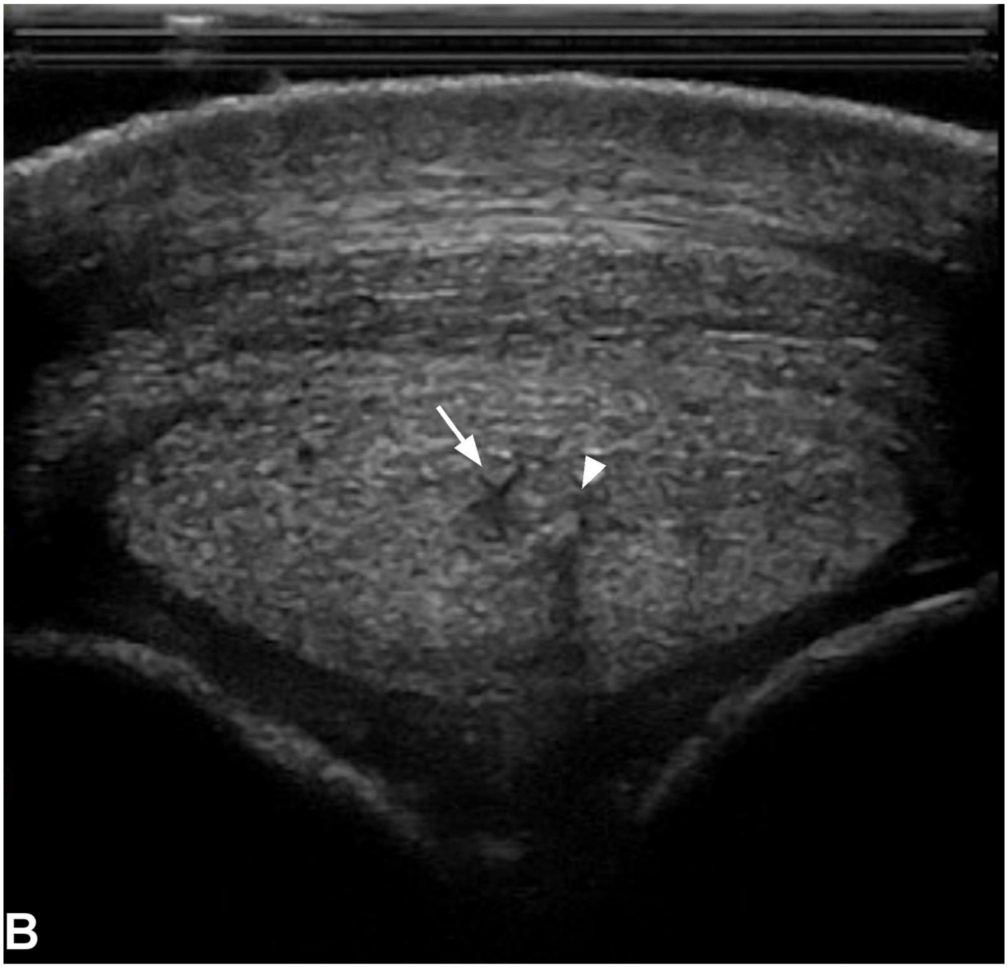

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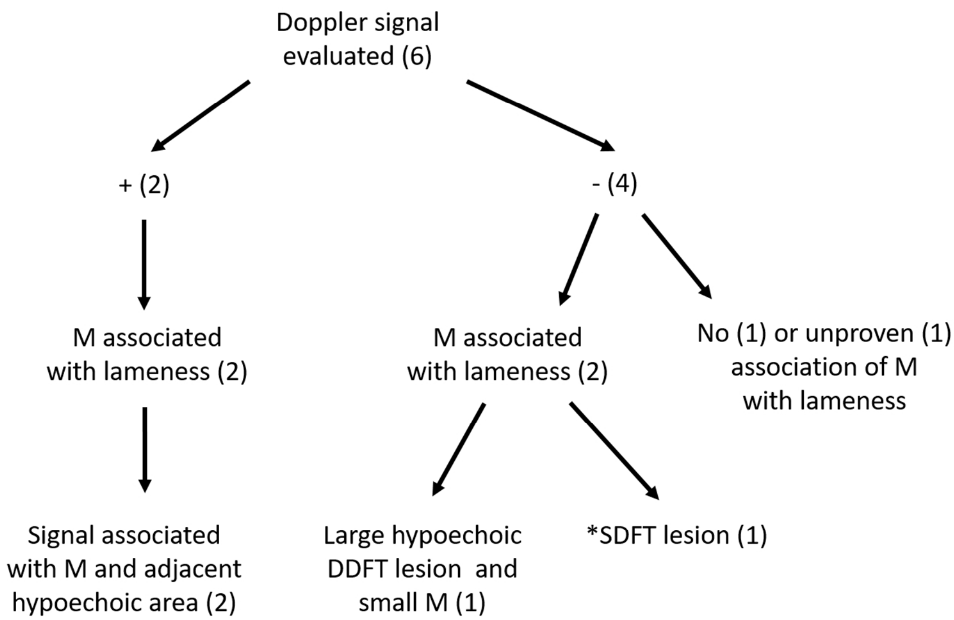
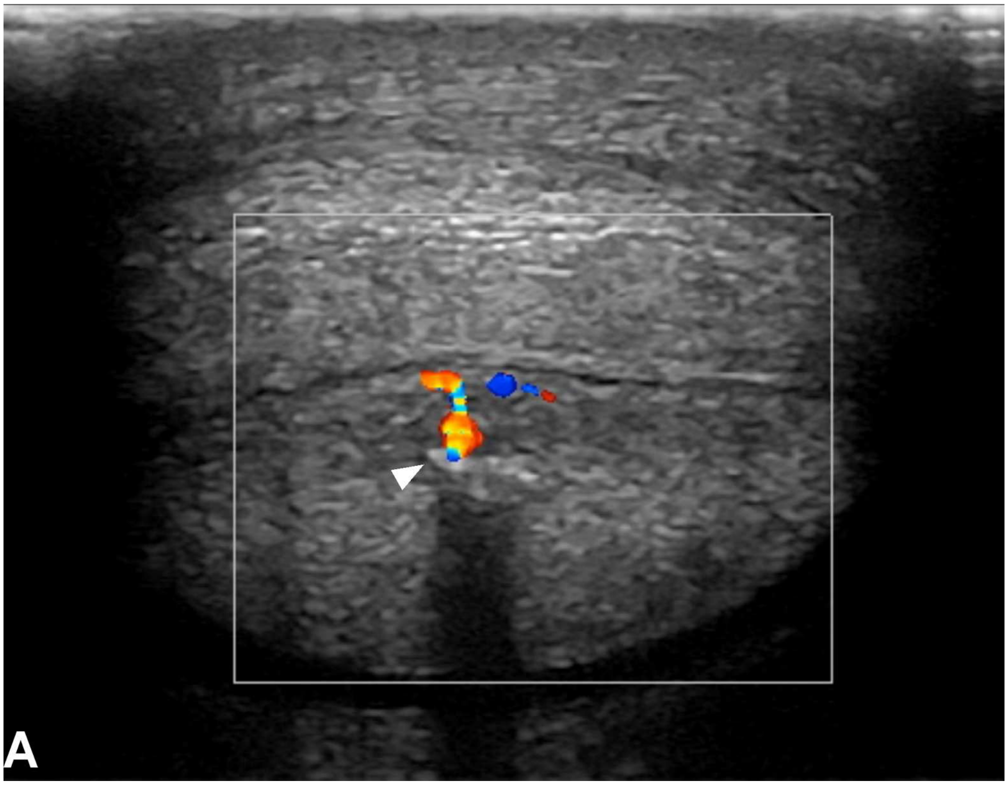


Overview of 6 limbs with deep digital flexor tendon (DDFT) mineralisation (M) evaluated for colour Doppler signal. + = positive Doppler signal in the affected tendon; - = absence of Doppler signal in the affected tendon; (number of limbs). Association of mineralisation with lameness was based on positive digital flexor tendon sheath analgesia. *SDFT = intrathecal superficial digital flexor tendon lesion which demonstrated Doppler signal.

235x150mm (144 x 144 DPI)



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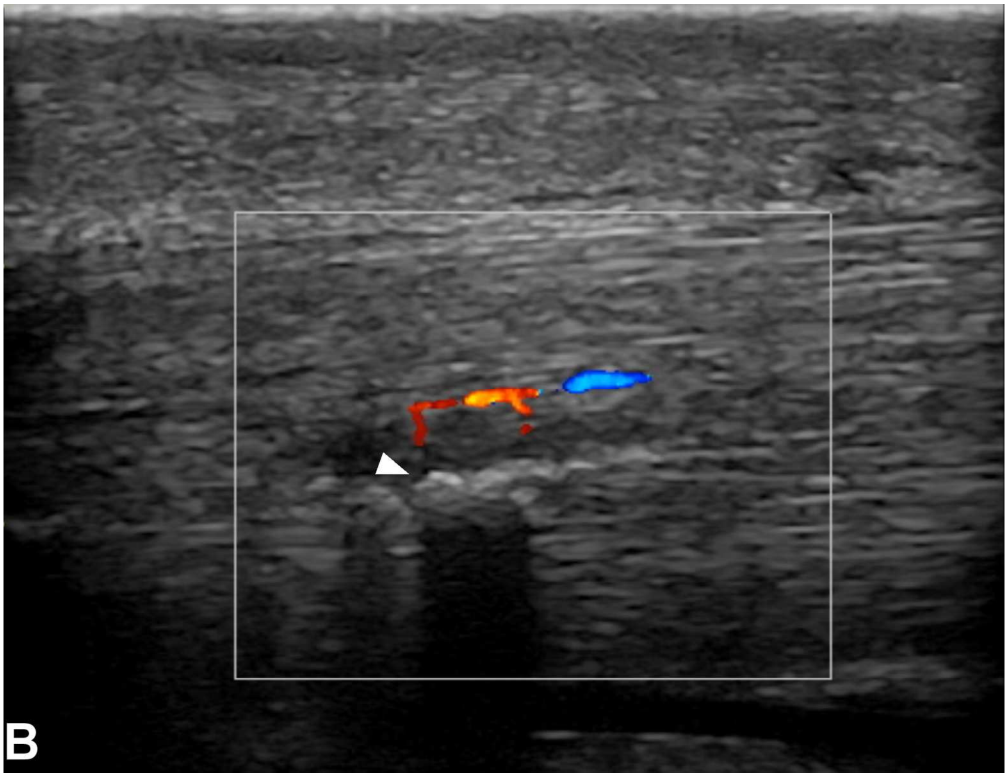

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