

1 **Case Report**

2 **Quadrilateral suspensory and straight sesamoidean ligament calcifying desmopathy in an Arabian**
3 **mare**

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12 **Keywords:** horse; desmopathy; suspensory ligament; degenerative; calcifying

13 Summary

14 A 5-year-old Arabian mare was referred for the investigation of recent behavioural change, generalised
15 stiffness, and owner-reported polyuria and polydipsia. Clinical examination revealed severe pain associated
16 with palpation along the entire length of the suspensory ligament (SL) and palmar soft tissues of the pastern
17 regions of both forelimbs. Radiographs of the distal forelimbs demonstrated marked radiopaque striations
18 within the straight sesamoidean ligaments (SSL), and less severe but similar changes in the regions of the
19 SL branches. Ultrasonography of both distal forelimbs revealed multifocal, hyperechoic lesions within the
20 SSLs. The SL bodies and branches were enlarged and had mixed echogenicity on ultrasound scan. The horse
21 was euthanased at the owner's request and submitted for post-mortem examination. Relevant gross
22 findings were restricted to the SLs and SSLs of all four limbs, all of which contained severe mineralisation
23 and irregularly arranged fibres. Histological examination revealed severe, multifocal to coalescing,
24 dystrophic mineralization with cartilaginous and osseous metaplasia, which suggested a primary calcifying
25 desmopathy affecting all four SLs and SSLs. Clinical findings in the case reported here were similar to that
26 of degenerative suspensory ligament desmitis (DSLDD) previously reported by many authors, but diagnostic
27 imaging and histopathological findings were significantly different.

28 Introduction

29 Mineralisation (ossification or calcification) is one of several possible histological features of desmopathy
30 and tendinopathy (O'Brien *et al.* 2012), but is rarely reported in equines. It is usually associated with a
31 previous episode of trauma, which may be iatrogenic in nature (O'Brien *et al.* 2012). In humans, pain is
32 reported to be associated with tendon mineralisation in the rotator cuff and other sites (O'Brien *et al.* 2012),
33 and we assume the same would be the case for *equidae*.

34 Degenerative suspensory ligament desmitis (DSLDD) is a debilitating condition of *equidae*, over-represented
35 in Peruvian Pasos, regardless of age, sex, or athletic function. Affected horses can have multi-limb pathology,
36 and often have pronounced lameness and pain on palpation of the SLs (Mero and Pool 2002; Mero and
37 Scarlett 2005; Miller and Juzwiak 2010; Xie *et al.* 2010). Horses with DSLDD are reported to have limited
38 response to treatment, including analgesics, stall confinement and rest (Mero and Pool 2002).

39 DSLDD has also been reported in older Arabians, American Saddlebreds, Quarter Horses, Thoroughbreds, and
40 some European breeds that are intensively worked or have sustained a prior suspensory desmitis (Halper
41 *et al.* 2006). The condition in these breeds is usually only bilateral (Dyson and Genovese 2011b). A single

42 case report by Miller and Juzwiak (2010) also described an unusual case of bilateral hindlimb DSLD in a 3-
43 month old Standardbred foal with an acute SL rupture.

44 Histopathology of DSLD-affected SLs typically shows degeneration and swelling of collagen bundles within
45 the ligaments, which gradually coalesce and fibrose with disease progression (Mero and Scarlett 2005;
46 Halper *et al.* 2006).

47 This case report details an unusual case of suspected quadrilateral primary calcifying quadrilateral
48 suspensory and straight sesamoidean ligament desmopathy, clinically similar to DSLD, but with different
49 ultrasonographic, radiological and histological findings due to marked calcification and ossification within
50 the affected ligaments.

51 **Case details**

52 ***Case history and physical examination***

53 A 5-year-old Arabian mare, kept at grass, was referred with a history of polyuria (PU), polydipsia (PD), a
54 change in temperament, and stallion-like behaviour. Marked stiffness was exhibited after rising from
55 recumbency. The referring veterinary surgeon suspected a granulosa-theca cell tumour of the left ovary and
56 the mare was referred for further investigation and possible ovariectomy.

57 On admission, the mare had a body condition score of 2/5 (Carroll and Huntington 1988) and normal vital
58 parameters. Trans-rectal ultrasonographic examination of the uterus and ovaries revealed normal sized
59 ovaries with multiple follicles of up to 2.5cm in diameter. Anti-Mullerian hormone levels were mildly
60 elevated at 3.2 ng/ml (reference range 0.22 – 2.94 ng/ml), but this result was considered clinically
61 insignificant, given the atypical history and normal ultrasonographic findings. It was therefore deemed
62 unlikely that the mare had a granulosa-theca cell tumour, and further investigations were performed.

63 Water intake was measured over a three-day period and was found to be within normal limits, despite PU
64 and PD being reported. Routine haematology and serum biochemistry showed no significant abnormalities.

65 The mare had a potterly gait at walk, was reluctant to trot and was bilaterally lame in front (right > left). She
66 was noted to be lying down for extended periods of time and was reluctant to move around the stable.

67 Digital pulses were within normal limits and the mare showed a negative response to hoof testers. Palpation
68 of the forelimb SL bodies, SL branches and soft tissues in the palmar pastern regions elicited severe pain
69 responses. It was extremely difficult to draw meaningful conclusions from hind limb palpation because
70 of the horse's temperament. There was a marked increase in lameness after distal limb flexion of all four
71 limbs.

72 **Radiographs**

73 Standing lateromedial radiographs of the distal limbs revealed no osseous abnormalities; however, multiple
74 radiopaque striations along the path of both SSLs in the forelimbs were visible. Less radiopaque changes
75 were also noted in the area of the SL branches just proximal to the fetlock (**Fig 1**). Similar mineralisation
76 was not seen in radiographs of the distal hindlimbs.

77 **Ultrasound examination**

78 A 12 MHz linear array transducer was used to examine the digital flexor tendons, SLs and SSLs of both
79 forelimbs. There was mild enlargement of the SL bodies (maximal cross sectional area 1.4cm², reference
80 range for Arabian horses 1.0 – 1.2cm²) visible on transverse (**Fig 2a**), and longitudinal (**Fig 2b**) views.
81 Numerous hypoechoic areas were present within the body of the SL with generalised hypoechogenicity and
82 loss of fibre pattern involving large portions of the ligament (**Fig 2b**). Multifocal, hyperechoic foci were
83 present in both left and right SSLs of the forelimbs (**Fig 3**).

84 **Outcome**

85 A non-steroidal anti-inflammatory drug (NSAID), meloxicam (Metacam 0.6 mg/kg SID PO)¹, was
86 administered in an attempt to alleviate the significant musculoskeletal pain. No improvement was seen after
87 five days and the owner elected to have the mare euthanased on humane grounds. The carcass was referred
88 to the University of Nottingham Veterinary Pathology Service for post-mortem evaluation.

89 **Post-mortem findings**

90 **Gross findings**

91 Relevant gross findings were restricted to the SLs and SSLs of all four limbs. The affected ligaments had
92 normal external surfaces, but changes could be seen on their cut surfaces. On transverse section, all four SLs
93 (proximal part, body and branches) and SSLs felt gritty and showed irregularly arranged fibres. Multifocal
94 pinpoint white foci of 0.5-1mm in diameter were seen on transverse incision, while the longitudinal incision
95 revealed severe white striations (mineralization) within the ligaments (**Fig 4**). Examination of all 4 fetlock
96 joints was unremarkable.

97 **Histological examination**

98 Representative tissue samples of liver, spleen, kidneys, ovaries, pituitary gland, SLs and SSLs were trimmed
99 and routinely processed for histological examination. Relevant histological changes were observed
100 primarily in the SLs and SSLs of all four limbs. Up to 90% of the SL and SSL were extensively mineralized,
101 characterised by abundant, finely granular, extracellular deposits within the ligament fibres. These deposits

102 stained dark purple with haematoxylin and eosin (HE) and dark-brown to black with von Kossa stain,
103 indicating mineralised granular deposition (**Fig 5**). Multifocally, islands of cartilaginous metaplasia with
104 central bone formation (endochondral ossification) were also noted within the ligaments. The
105 interfascicular connective tissue within the most severely affected areas showed minimal multifocal
106 changes, characterized by low numbers of individual medium-sized arteries with intimal mineralization and
107 mild multifocal vascular congestion of small blood vessels. Besides the above described mineral, cartilage
108 and bone deposits, there was no evidence of any other extracellular matrix deposition (e.g. proteoglycans
109 or fibrosis). Given the lack of inflammatory changes or evidence of trauma on any samples examined, the
110 observed lesions were suspected to be of a degenerative nature.

111 **Discussion**

112 Ligament and tendon mineralisation may be due to calcification or ossification (O'Brien *et al.* 2012). The
113 horse in this report had both abnormal calcium deposition and evidence of endochondral ossification in the
114 SLs and SSLs. We believe that this type of severe quadrilateral mineralising desmopathy has not been
115 described previously in equine literature. Whilst the mare described in this case showed many clinical
116 similarities to DSLD, including multi-limb involvement, severe pain evoked by palpation of SLs, enlarged
117 SLs, positive response to fetlock flexion tests, increased recumbency time, lameness, and reluctance to move
118 (Mero and Pool 2002), diagnostic imaging and histopathological findings of the affected ligaments were
119 different from the typical findings of DSLD (Mero and Pool 2002; Miller and Juzwiak 2010).

120 Aetiology of mineralisation of the SLs and SSLs in this case is unknown. The mare was a 5-year-old
121 homebred pasture pet, and had not undertaken any significant athletic pursuit in her lifetime. Quadrilateral
122 involvement and lack of inflammation seen on histopathological examination makes a traumatic aetiology
123 unlikely, and a primary degenerative desmopathy is suspected.

124 Tendon mineralisation is reported to be a cause of pain and tendon weakness in both humans and equines
125 (O'Brien *et al.* 2012; Dyson 2011a), and is associated with tendon failure, presumably because it impacts on
126 the biomechanical function of the tendon (O'Brien *et al.* 2012; Dyson and Genovese 2011b). It was unclear
127 how long the mineralisation process had been present, or how quickly it progressed, but the mare had been
128 showing clinical signs for only 6 weeks. It would be possible that as she was turned out and not in regular
129 work, that she may have had a level of undetected lameness for much longer than the reported time period.
130 Diagnostic imaging and histopathological findings of the affected ligaments in this case were significantly
131 different from the typical findings of DSLD (Mero and Pool 2002; Miller and Juzwiak 2010).

132 Ultrasonographic changes observed within a DSLD-affected SL include enlargement of the ligament and
133 diffuse loss of echogenicity and fibre pattern (Mero and Scarlett 2005; Miller and Juzwiak 2010). In the case
134 reported here, the SLs had a diffuse loss of echogenicity and fibre pattern with numerous focal hypoechoic
135 areas within the ligaments. In addition, widespread hyperechoic foci were seen within the SSLs. Some of
136 these foci created shadowing artifact, indicating mineralization of the ligaments.

137 Heterotopic ligament mineralisation is an uncommon feature of DLSD, where the hallmark histological
138 findings are abnormal proteoglycan (PG) accumulation in SLs, and in other connective tissues including the
139 superficial and deep digital flexor tendons, patellar and nuchal ligaments, aorta, coronary arteries and
140 sclerae (Halper *et al.* 2006). An alternative aetiology was proposed by a more recent study by Schenkman
141 *et al.* (2009), who concluded that abnormal PG deposition in DSLD-affected ligaments likely developed as a
142 result of disease progression and was not the cause. Small foci of cartilage calcifications are occasionally
143 found in the SLs of advanced cases of DSLD (Halper *et al.* 2006), but the SLs in the case reported here were
144 extensively affected. The SL and SSLs of all limbs in this case were extensively mineralised with frequent
145 cartilaginous metaplasia and islands of endochondral ossification; these changes appeared to be
146 progressive and irreversible, and were associated with overt lameness and marked pain on palpation.

147 There was no evidence of abnormal PG deposition in the tendons, ligaments, or other anatomical structures
148 examined histologically in this case report, and as such, this was dissimilar to previously described cases of
149 DSLD. Given the range of changes observed within the affected ligaments, the term 'calcifying desmopathy'
150 would best describe the case reported here.

151 Whilst unilateral or bilateral tendon injury is a very common disorder in horses, tendon or ligament
152 mineralisation is a rare event. In contrast, tendon mineralisation is found relatively commonly (2.7 - 22%
153 prevalence) after rotator cuff injury in human beings (Oliva *et al.* 2011). Other less commonly affected sites
154 include the Achilles tendon, bicep brachii tendon, extensor pollicis longus tendon, quadriceps tendon,
155 anterior cruciate ligament and medial collateral ligament (O'Brien *et al.* 2012). In humans, tendon
156 mineralisation also arises after surgical trauma, but can also occur as a feature of a primary tendinopathy
157 or desmopathy (O'Brien *et al.* 2012; Lafuente *et al.* 2009). In horses, tendon mineralisation is reported
158 anecdotally after injection of corticosteroids either into the digital flexor tendon sheath (Dyosn 2011a) or
159 directly into the tendons themselves (usually for treatment of core lesions). Several reports have also
160 documented the development of biceps brachii tendon calcification or ossification in cases of chronic biceps
161 brachii tendinitis in horses (Gillis and Vatistas 1997; Meagher *et al.* 1979). Seignour *et al.* (2011) reported

162 that there was an association between fibrosed, mineralized palmar or plantar distal digital annular
163 ligament and chronic ligament injury. The horse in this case had no previous history or histological evidence
164 of trauma or inflammation, and similarly no history of iatrogenic intervention that could have induced the
165 changes seen at post-mortem.

166 A study by Baird and Kang (2009) demonstrated that NSAID administration reduced the development of
167 approximately 60% of heterotopic ossification in traumatized human tissue. This indicated that
168 inflammation had an important role in heterotopic ossification development. Other mechanisms for
169 heterotopic ossification within tendons (without necessarily having pre-existing injury or inflammation)
170 that are proposed in human medicine and could potentially be relevant to this case include tendon
171 underuse, genetic factors, tissue hypoxia, hormonal, and endocrine disorders (O'Brien *et al.* 2012). All
172 presumably affect bone morphogenetic protein (BMP) production within affected tendons (O'Brien *et al.*
173 2012).

174 Ingestion of plants containing toxic levels of vitamin D-like compounds, such as day jasmine (*Cestrum*
175 *diurnum*) in Florida (Krook *et al.* 1975), or nightshade (*Solanum glaucophyllum*) in Argentina and Brazil
176 (Worker and Carrillo 1967), were reported to cause hypercalcaemia and widespread metastatic tissue
177 calcification in horses. The mare in this case report had normal blood calcium levels, and did not show any
178 evidence of metastatic calcification in any of the organs and tissues examined histologically. The ligament
179 abnormalities observed in this case could have been due to a toxic insult, but of course this cannot be ruled
180 in or out definitively.

181 Given the severe and extensive changes seen at post-mortem and on histological examination, we believe
182 that euthanasia was the correct decision for this case. Although the duration of attempted medical treatment
183 was quite short, the mare was frequently recumbent and showed significant signs of distress after rising
184 from recumbency; as such her quality of life was deemed to be poor by both the owner and ourselves. There
185 is currently no cure for equine DSLD. It is a progressively debilitating disease with a poor prognosis, and
186 many cases are similarly euthanased on humane grounds.

187 **Manufacturer's address**

188 ¹Boehringer Ingelheim Limited, Bracknell, Berkshire, United Kingdom

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