**Original Article**

**Histological examination of the interspinous ligament in horses with overriding spinous processes**

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**Abstract**

The equine interspinous ligament (ISL) consists of an oblique crossing arrangement of collagenous bundles which are thought to counteract the tensile and rotational forces of distraction between the spinous processes (SPs) in the caudal thoracic and cranial lumbar spine. The aim of this controlled histological study was to assess the structural anatomy and innervation of the ISL in horses with clinically significant overriding (dorsal) SPs (ORSPs) and to compare the findings with the ISL of normal horses. Samples of the ISL were obtained from 10 horses that underwent subtotal ostectomy for treatment of ORSPs. Control samples were obtained from horses without spinal pathology. Histological staining of ISL sections with haematoxylin and eosin was performed to assess the morphology of the ligaments and with Alcian blue-periodic acid-Schiff to determine the proteoglycan and glycosaminoglycan content. Immunohistochemistry for S100 was performed for quantitative evaluation of nerves within the ISL.

The ISL in horses with ORSPs had an altered collagen fibre alignment and arrangement of the ligamentous layers when compared to healthy controls. A significant increase in fibrocartilaginous tissue with evidence of fibrocartilaginous metaplasia was detected (*P* = 0.001). The number of nerves in the ISL samples was significantly higher in horses with ORSPs than in controls (*P* = 0.017). Structural alterations of the ISL, including loss of fibre alignment and fibrocartilaginous metaplasia, are associated with ORSPs in the equine thoracolumbar spine. In addition, an increase in innervation of the ISL in horses with ORSPs, compared to normal, may explain the thoracolumbar pain experienced by some horses with ORSPs.

*Keywords:* Equine; Fibrocartilaginous metaplasia; Innervation; Spinal ligament

**Introduction**

Jeffcott (1980) first described overriding (dorsal) spinous processes (ORSPs) as one of the most common causes of thoracolumbar pain in the equine athlete. ORSPs have been reported in asymptomatic riding horses, as well as in horses with thoracolumbar pain (Walmsley et al., 2002; Erichsen et al., 2003, 2004; Wennerstrand et al., 2004). ORSPs are defined as narrowing of the space between two thoracolumbar spinous processes to less than 4 mm (Zimmerman et al., 2012).

The aetiology of ORSPs is not clearly understood. A radiographic survey performed in Warmblood foals did not show any evidence for ORSPs, suggesting that the narrowing of the interspinous space is unlikely to have a congenital aetiology in this breed (Sinding and Berg, 2010). Clinical signs of thoracolumbar pain can range from non-specific signs of poor performance to bucking behaviour under saddle. A pain response upon palpation of the thoracolumbar spine and the epaxial musculature, and/or reduced kinematic measures, including dorsoventral and lateral movement of the spine, are commonly observed (Wennerstrand et al., 2004; Stubbs et al., 2006). Increased radiopharmaceutical uptake in the area of the SPs on nuclear scintigraphy and a positive response to local infiltration with anaesthetic agents are useful indicators for the clinical significance of existing ORSPs (Jeffcott, 1980; Zimmerman et al., 2011).

Radiographic abnormalities detected on lateral-lateral radiographs of horses with ORSPs include periosteal reactions, increased radio-opacity of subcortical bone, osteolytic cyst-like lesions, malformation of SPs and formation of pseudo-articulations or fusion between SPs (Jeffcott, 1975; Jeffcott, 1980; Zimmerman et al., 2012). However, radiographic and scintigraphic evidence for ORSPs is not necessarily correlated with clinical signs of thoracolumbar pain (Erichsen et al., 2004; Zimmerman et al., 2011, 2012).

The structure and innervation of the equine interspinous ligament (ISL) have been investigated in detail. The equine ISL is composed of an oblique crossing arrangement of collagenous bundles, which are thought to counteract tensile and rotational forces of distraction between the SPs in the caudal thoracic and cranial lumbar spine (Ehrle et al., 2017). As in human beings and laboratory animals, a dense sensory innervation of the supraspinous and interspinous ligaments has been identified in the equine thoracolumbar spine (Jiang et al., 1995; Scapinelli et al., 2006; Tesarz et al. 2011; Ehrle et al., 2017). An increase in nociceptive input to the lumbar dorsal horn neurons, with an increase in density of nociceptive fibres, has been demonstrated in laboratory animals following induced inflammation in the area of the thoracolumbar spine (Hoheisel and Mense, 2015; Hoheisel et al., 2015). Clinical studies have suggested that ORSPs in horses may be associated with desmitis of the ISL or a compartment syndrome in the area of the interspinous space (Fonseca et al., 2006; Coomer et al., 2012).

The aim of this study was to investigate potential alterations to the ISL associated with ORSPs in the equine thoracolumbar spine. It was hypothesised that the ISL undergoes structural changes in horses with ORSPs and that the soft tissues adjacent to the ORSPs exhibit upregulation of sensory innervation when compared to horses without thoracolumbar pathology.

**Material and methods**

*Animals and samples*

Samples were obtained from 10 horses that underwent subtotal ostectomy as a surgical treatment for ORSPs. Informed owner consent for tissue retention was obtained and approval for the study was given by the local committee on research ethics (Approval number VREC261; date of approval 17 December 2014). All horses were presented for investigation of signs of poor performance or behavioural changes and thoracolumbar pain. To be included in the study, horses had to show a significantly decreased pain response after local infiltration of the affected area with an anaesthetic agent, have evidence for ORSPs on radiographic examination and show increased, focal radiopharmaceutical uptake on scintigraphic examination (Zimmerman et al., 2012). In addition, all horses involved in the study had an unsatisfactory clinical response to conservative management of 3-6 months duration, including perilesional medication with methylprednisolone (maximum dose of 120 mg Depo-Medrone, Pfizer), controlled exercise (starting with hand walking and subsequently continuing with an individual lungeing or long-reining programme) and regular physiotherapy (with attendance of a qualified physiotherapist two to four times a month).

Lateral-lateral radiographs of the thoracolumbar SPs were obtained in all cases. To facilitate orientation on the radiographs, radiodense markers, which appeared radio-opaque on the radiographs, were placed over the eighth and sixteenth thoracic and the second lumbar vertebrae. The sites of the markers were clipped for subsequent identification of affected SPs. ORSPs were defined as narrowing of the space between two spinous processes to less than 4 mm. The radiographs were graded from 0 to 7 according to a published grading system (Zimmerman et al., 2012).

Horses were injected intravenously (IV) for nuclear scintigraphy with 99m technetium methylene diphosphonate (99mTc MDP) at 10 MBq/100 kg following lungeing exercise for 15-20 min to promote optimal distribution of the radioisotope. The diuretic furosemide (Dimazon 5%; 0.5 mg/kg body weight IV, MSD Animal Health) was administered 90 min after injection of 99mTc MDP, followed in 60 min by scintigraphic examination. Images were obtained on a gantry-mounted, digital rectangular large field of view gamma camera (Bartec Technologies) and then processed using a nuclear medicine software for interpretation (Hermes Medical Solutions). Left and right lateral 60° ventral images of the thoracolumbar spine were obtained in all horses. Increased radiopharmaceutical uptake was graded as mild, moderate or severe (Erichsen et al., 2003).

For diagnostic analgesia, ORSPs were located with the assistance of the previously described radiodense markers and clip marks. Following aseptic preparation and local analgesia of the skin, a 21 G x 50 mm needle was advanced adjacent to the affected interspinous space on both sides. A total of 10 mL of mepivacaine hydrochloride 2% (Intra-Epicaine, Dechra Veterinary Products), 5 mL on the right and left side each, was injected per affected site to desensitise the area.

Subtotal ostectomy of the affected SPs was performed under standing sedation and local anaesthesia in all horses included in the study. Horses received procaine benzylpenicillin (12 mg/kg body weight intramuscularly, IM, Depocillin, MSD Animal Health), gentamicin (6.6 mg/kg body weight IV Genta-Equine, Dechra Veterinary Products) and flunixin meglumine (1.1 mg/kg body weight IV Mefosyl, Zoetis) prior to surgery. A dorsal midline incision was made over the affected SPs and the suspraspinous ligament was divided longitudinally. The multifidus and longissimus dorsi muscles were separated from their attachments to the SPs and the ISL cranial and caudal to the affected SPs was incised sharply. A pneumatic oscillating saw was used to resect either the cranial only, or the cranial and the caudal portion, of the affected SPs, depending on the number and location of affected spaces. The supraspinous ligament was subsequently re-apposed and the incision was closed in two layers (Walmsley et al., 2002; Perkins et al., 2005; Brink, 2014; Jacklin et al., 2014). The resected portions of the affected SPs, including the attached ISL and myofascial attachments, were fixed in 10% neutral buffered formalin for 48-72 h before being trimmed for paraffin embedding and tissue sectioning (4 µm) for histological examination.

Control samples originating from a previous study of the structure and innervation of the ISL in horses without evidence for spinal pathology were used as a comparison group (Ehrle et al., 2017). These samples of ISL were obtained from 10 skeletally mature horses, including six geldings and four mares, aged 4-18 years. The breeds represented were Thoroughbred (*n* = 3), Irish sports horse (*n* = 3), Warmblood (*n* = 1), Cob (*n* = 1), Arabian (*n* = 1) and Lusitano (*n* = 1). Horses included in the study were humanely destroyed for reasons unrelated to thoracolumbar pathology. Horses with radiographic evidence of spinal pathology or pathological changes noted during dissection were excluded from the study. An ISL tissue sample was harvested from each interspinous space between the 15th thoracic and the first lumbar vertebrae in each specimen within 2 h following euthanasia. The sections were cut immediately adjacent to the bony margins of the SPs from the level of the supraspinous ligament to the most ventral part of the ISL at the intervertebral junction.

*Histomorphological and immunohistochemical study*

The ISL was cut adjacent to the bony margins of the resected portion of the SPs. ISL tissue was harvested from the most dorsocranial and dorsocaudal aspects of the resected SP wedge to the most ventral part of the resected bone. In cases where the cranial aspect of the SP was overlapping or fused with an adjacent SP, ISL tissue was collected just ventral to the affected area. One slide per SP interface, containing multiple transverse and sagittal sections of ISL, was prepared for histological examination. A total of four slides, with tissue from four different interspinous spaces affected by ORSPs, were examined per horse.

Staining with haematoxylin and eosin (H&E) was performed for histomorphological evaluation of the ISL sections. On the basis of the morphological appearance, tissue was categorised as ligamentous, adipose, muscular or fibrocartilaginous. Digital photomicrographs were prepared (Nikon Eclipse i80 equipped with Nikon DS 5mc digital camera; 1600x1200 pixels) from which the cross-sectional area of the different tissue categories in each slide was determined (Image J, National Institute of Health[[1]](#footnote-1)) and the percentage area of each tissue was calculated (Ehrle et al., 2017).

Alcian blue-periodic acid-Schiff (Alcian-PAS) staining was performed to highlight the proteoglycan and glycosaminoglycan content in the histological samples. The percentage area of positive uptake of the Alcian-PAS stain (blue staining) was calculated relative to the non-staining tissue (pink staining), excluding the background, using an automated image analysis software (Orbit image analysis, Idorsia Pharmaceuticals) in each of the slides of horses affected with ORSPs, as well as in control horses.

IHC using anti-S100 antibody (rabbit polyclonal anti-S100; Dako; dilution 1:100) was performed in order to quantify nervous tissue, following a previously described protocol (Ressel et al., 2015). Nerves were ranked as small (single nerve fibre), medium (bundle of two to five nerve fibres) or large (bundle of ≥ six nerve fibres) (Ehrle et al., 2017). The number of nerve fibres in collagenous, adipose, muscular and cartilaginous tissue was documented and the nerve density within each tissue type was calculated (nerves/mm2) for each individual tissue section.

*Data analysis*

Data was recorded in Excelversion 2010 (Microsoft) and analysed using Minitab 17 and SAS 9.4 (SAS Institute) software. Descriptive statistics were performed to assess the composition of the ISL in horses with ORSPs and the control samples. Differences in nerve distribution were calculated in the ISL sections of healthy horses and horses with ORSPs using the Kruskal-Wallis test. The difference between the area of fibrocartilaginous metaplasia and unaffected tissue, and the content of adipose tissue, was investigated using the Mann-Whitney *U* test.

A linear mixed regression model was used to investigate the effects of disease, type of tissue (ligament, fat or muscle) and nerve size on the number of nerves per cross sectional area (mm2). The data were hierarchically structured as ‘tissue’ within ‘interspinous space’ and ‘interspinous space’ within ‘horse’. ‘Horse’ and ‘interspinous space’ within ‘horse’ were used as random effects. Disease status (positive/negative), tissue (ligament, fat or muscle), nerve size (small, medium or large) and the interaction between tissue type and nerve size were determined as fixed effects. In order to receive normally distributed and homoscedastic residues, the dependent variable (cartilaginous tissue or nerves/mm2) was transformed to logarithmic values on the basis of 10 and a Box-Cox transformation with lambda-2 was performed. Lambda was selected by maximizing the profile log-likelihood function. Model construction was carried out with the use of -2 Log Likelihood and Akaike’s information criterion. In the final model, restricted maximum likelihood estimations were used to investigate residuals. Influence statistics included Cook’s D and leverage. Additionally, the intraclass correlation coefficient (ICC) for the hierarchical levels ‘horse’ and ‘interspinous space’ was calculated. *P* values < 0.05 were considered to be statistically significant.

**Results**

Samples were obtained from six geldings and four mares, aged 5-15 years (mean 9.8 years). The breeds represented were Thoroughbred (*n* = 3), Warmblood (*n* = 3), Irish sports horse (*n* = 3) and Irish draught horse (*n* = 1). Cranial wedge ostectomy was performed on 14 SPs in three horses; subtotal ostectomy was performed on 32 SPs in seven horses between the 14th thoracic and the first lumbar vertebrae (Table 1).

 H&E staining of ISL sections from horses with ORSPs confirmed marked structural changes in the diseased ligament. The ISL of horses without evidence of SP pathology has previously been shown to consist of multiple ligamentous layers with an adipose core between adjacent SPs (Ehrle et al., 2017). It was not possible to distinguish between these different layers of the ISL in the ORSP sections. In the diseased tissue, the normal adipose core was absent and the presence of adipose connective tissue between ligamentous layers was significantly reduced (*P* = 0.001). Instead of a continuous fibre direction, the ligamentous tissue contained multiple areas of dense cartilaginous tissue (Fig. 1).

Alcian-PAS staining confirmed a significantly higher content of proteoglycan and glycosaminoglycan in samples from horses with ORSPs compared with control samples (*P* = 0.001). The median area of metaplasia was 9.9% (range 2.4-32.2%) for ORSP samples and 0.1% (range 0-2.8%) for control samples (Fig. 2). Areas of fibrocartilaginous metaplasia with the presence of chondrocyte clusters (chondrones) were detected throughout all ISL sections of horses with ORSPs, but were only rarely seen in close proximity to the bony margin of the SPs in the control samples (Fig. 1).

The quantitative evaluation of nervous tissue (S-100 immunostaining) identified a significantly higher number (*P* = 0.017) of single, free nerve endings/mm2 in the ISL (ligament and fat component) obtained from horses with ORSPs when compared to the control samples (Table 2). However, only a small number of free nerve endings were detected in the cartilaginous tissue, whilst most small nerves were clustered at the margins of these metaplastic areas. A wide variation in the nerve density within the ISL was found between different horses and the different interspinous spaces (Fig. 3).

In the final linear mixed regression model, disease (*P* = 0.005), tissue type (*P* = 0.001), nerve size (*P* = 0.001) and the interaction between tissue and nerve size (*P* = 0.001) showed a significant influence on the number of nerves/mm2. There were significantly more small nerves than medium and large nerves in all three types of tissue. ICCs indicated that 40.1 % of the variance in the number of nerve fibres was due to differences between horses and 24.4 % of the variance was due to differences between the interspinous spaces.

**Discussion**

Histological examination of the ISL in horses with ORSPs identified the loss of ligamentous integrity and disruption of the normal anatomy, with evidence of fibrocartilaginous metaplasia throughout the ligament. In addition, a significant increase in the density of small nerves, most likely sensory nerves, was detected in the tissue samples from horses with ORSPs when compared to the control samples.

Fibrocartilaginous metaplasia is defined as the phenotypical transformation of fibroblasts into chondrocytes, with subsequent change in matrix production, which occurs as a response to chronic soft tissue irritation (Vogel, 2003). Compressive forces, shear forces and tensile stress lead to fibrocartilaginous metaplasia in tendons and ligaments in human beings and laboratory animals (Benjamin and Ralphs, 1998; Wren et al., 2000). Fibrocartilaginous metaplasia has been detected in tendons that wrap around bony pulleys in the human hand and ankle and has been associated with rotator cuff tendon pathology in human beings (Benjamin and Ralphs, 1998; Gigante et al., 2004; Varitimidis et al., 2016). In horses, fibrocartilaginous metaplasia has been identified in the superficial and deep digital flexor tendons (Crevier-Denoix et al., 1998; Blunden et al., 2009), the manica flexoria (Findley et al., 2017), the suspensory ligament (Lischer et al., 2006) and the collateral ligaments of the distal interphalangeal joint and the metacarpophalangeal/metatarsophalangeal joints (Dyson et al., 2008; Pohlin et al., 2014). Especially where ligaments or tendons run over bony prominences, compressive and tensile forces decrease the nutrient and oxygen supply within the tissue, which leads to an adaptive upregulation of proteoglycan content and alteration of these connective tissue cells to a cartilaginous phenotype (Gigante et al., 2004; Beck et al., 2011). Subsequent to fibrocartilaginous metaplasia, heterotopic mineralisation, including calcification or enchondral ossification, can occur (O’Brien et al., 2012; Magne and Bougault, 2015).

The movement of the equine thoracolumbar spine during locomotion includes flexion and extension, lateral bending and axial rotation, with forces of distraction affecting the ISLs between the SPs, especially when the horse is working in canter (Haussler et al., 2001; Faber et al., 2001; Greve et al., 2017). The distraction and compression of the ISL fibres between the SPs during movement could explain the development of fibrocartilaginous metaplasia. The decreased plasticity of the affected ISL fibres due to fibrocartilaginous metaplasia might have an effect on the elastic properties of the ISL and promote narrowing of the interspinous space, with subsequent mineralisation of ISL tissue. As described for other ligaments, such as the collateral ligaments and the deep digital flexor tendon, fibrocartilaginous metaplasia often starts at the enthesis (the connection between the ligament/tendon and bone) (Gardner, 1992). A similar mechanism may initiate fibrocartilaginous metaplasia in the ISL, since initial radiographic changes of ossification in the area of the ISL are usually located close to the enthesis of the ISL on the SPs (Zimmerman et al., 2011, 2012).

Dense sensory innervation of the soft tissues adjacent to the SPs, including the ISL, the supraspinous ligament and the thoracolumbar fascia, has been shown in human beings, laboratory animals and horses (Jiang et al., 1995; Scapinelli et al. 2006; Tesarz et al., 2011; Ehrle et al., 2017). Greater intensity and duration of pain was observed when pain was induced in the ISL compared to injection of the paraspinal muscles in human beings (Tsao et al., 2010). Recent investigations suggest that induced inflammation in the thoracolumbar spine region increases the nociceptive input to the lumbar dorsal horn neurons, with a subsequent increase in the density of nociceptive fibres in rats (Hoheisel and Mense, 2015; Hoheisel et al., 2015). Similarly, a significant increase in the total number of nerve fibres, most likely to be sensory nerves, was detected in this investigation of horses with thoracolumbar pain related to ORSPs.

The results of this study suggest that, despite the marked increase in density of nerves in horses with ORSPs when compared to the control horses, a wide variation in the density of nerves exists between different horses with ORSPs (Fig. 3). ORSPs can be an incidental finding in sports horses performing normally at a high level, but can lead to severe signs of pain in other horses (Jeffcott, 1979; Townsend et al., 1986; Walmsley et al. 2002; Erichsen et al., 2004). The inconsistent degree of upregulation of nociceptive fibres in the ISL of horses with ORSPs might explain the variation observed between cases, with more severe discomfort experienced by some horses when compared to others with similar radiographic abnormalities.

A limitation of this study is that the degree of pain caused by ORSPs and the duration of the pathological process is difficult to determine. ORSPs are diagnosed radiographically and an increase in bone turnover in the area of the SPs can be confirmed using gamma scintigraphy. However, the degree of pain associated with ORSPs does not generally correlate with the severity of findings detected on diagnostic imaging (Erichsen et al., 2004; Zimmerman et al., 2011, 2012). Only horses that were showing obvious clinical signs of discomfort and did not respond to medical management of thoracolumbar pain were included in the study; however, pathology of closely associated structures, including the articular process joints, the supraspinous ligament and the adjacent epaxial musculature, might have contributed to the horses’ level of discomfort. An additional limitation is that the sample collection in horses with ORSPs was performed during a non-experimental surgical procedure in horses that had been injected previously with corticosteroids.

**Conclusions**

Structural changes in the ISL, including areas of fibrocartilaginous metaplasia and the loss of fibre alignment and ligamentous tissue, are associated with ORSPs in the equine thoracolumbar spine. Further work investigating the role of the ISL in the early pathogenesis of ORSPs is warranted. The significant and variable increase in innervation of the ISL might explain the inconsistent degrees of pain and clinical signs of thoracolumbar dysfunction experienced by horses with ORSPs.

**Conflict of interest statement**

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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**Table 1**

Details of horses with overriding (dorsal) spinous processes (SPs) included in the study.

|  |  |  |
| --- | --- | --- |
| Horse details | SPs affected | Surgical technique |
| Warmblood, male, 8 years | T14-L2 | Caudal wedge ostectomy T14Cranial wedge ostectomy T15-L1ISL desmotomy L1-L2 |
| Irish Sports Horse, male, 12 years | T14-L1 | Caudal wedge ostectomy T14Subtotal ostectomy T15-T18 |
| Warmblood, female, 12 years | T15-L1 | Subtotal ostectomy T16 + T18 |
| Thoroughbred, male, 11 years | T13-T17 + T18-L2 | Subtotal ostectomy T14 + T16 + T18Cranial wedge ostectomy L2 |
| Irish Sports Horse, female, 7 years | T15-L1 | Cranial wedge ostectomy T16-L1 |
| Warmblood, female, 15 years | T12-L2 | Caudal wedge ostectomy T12Subtotal ostectomy T14 + T16Cranial wedge ostectomy T18-L1ISL desmotomy L1-L2 |
| Thoroughbred, male, 13 years | T14-T18 | Cranial wedge ostectomy T15-T18 |
| Thoroughbred, female, 9 years | T15-L1 | Caudal wedge ostectomy T15Cranial wedge ostectomy T16-L1 |
| Irish Sports Horse, male, 6 years | T12-L4 | Subtotal ostectomy T13 + T15 + T17Cranial wedge ostectomy L1ISL desmotomy L2-L4 |
| Irish Draught Horse, male, 5 years | T15-L1 | Cranial wedge ostectomy T16-L1 |

ISL, interspinous ligament; T, thoracic vertebrae; L, lumbar vertebrae.

All horses had four or more interspinous spaces affected by the disease.

**Table 2**

Quantitative assessment of nerve distribution (nerve fibres/mm2) in the interspinous ligament (ISL) of horses with overriding spinous processes (ORSPs) and control horses a within ligament, fat or adjacent muscle.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | ISL of horses with ORSPs | ISL of control horses |
|  | Ligament | Fat | Muscle | Ligament | Fat | Muscle |
| Small nerves b | Median | 3.54 | 3.19 | 1.21 | 1.11 | 1.42 | 1.99 |
|  | Range | 0.65-20.18 | 0.45-16.25 | 0.0-9.52 | 0.03-11.80 | 0.0-14.72 | 0.05-6.59 |
| Medium nerves | Median | 0.39 | 0.78 | 0.30 | 0.11 | 0.30 | 0.11 |
|  | Range | 0.04-4.93 | 0.12-5.40 | 0.0-5.43 | 0.0-0.74 | 0.0-2.82 | 0.0-1.32 |
| Large nerves | Median | 0.01 | 0.12 | 0.09 | 0.01 | 0.10 | 0.17 |
|  | Range | 0.01-0.07 | 0.0-1.10 | 0.0-0.99 | 0.0-0.52 | 0.0-2.44 | 0.0-14.72 |

a Data for control horses were obtained from a published study investigating the structure and innervation of the equine ISL in horses without spinal pathology (Ehrle et al. 2017).

b Small, one nerve fibre; medium, two to five nerve fibres; large, ≥ six nerve fibres.

**Figure legends**

Fig. 1. Alcian blue-periodic acid-Schiff staining of a histological section of the interspinous ligament (ISL) from a horse with an overriding spinous process (ORSP) (A) and a healthy control horse (B) (original magnification 400x). In horses with ORSPs (A), there is loss of ligamentous integrity. The fibre arrangement is undefined and wide areas of the ligament exhibit fibrocartilaginous metaplasia with deposition of proteoglycans and glycosaminoglycans (blue stain). The cartilaginous tissue contained multiple chondrocyte clusters (inset). In healthy horses (B), the ISL consisted of ligamentous fibre bundles with a designated fibre direction. Scale bar = 20 μm.

Fig. 2. Box plots representing the percentage of Alcian blue-periodic acid-Schiff positive staining per cross sectional area (CSA, mm2) in histological sections of the interspinous ligament (ISL) from horses with overriding spinous processes (ORSPs) and healthy control horses. Black lines represent the median. Whiskers represent values outside the interquartile range. Extreme values are indicated by asterisks (\*). The proteoglycan and glycosaminoglycan contents were significantly higher in samples from horses with ORSPs when compared to the control samples.

Fig. 3. Distribution of small nerves per cross sectional area (CSA, mm2) in ligamentous tissue in histological sections of the interspinous ligament (ISL) from 10 horses with overriding spinous processes (ORSPs). A wide variation in nerve density was found in the ISL between different horses. Box plots represent the interquartile range of nerve distribution in horses with ORSPs. Black lines represent median values. Whiskers represent values outside the interquartile range.

1. See: <https://imagej.nih.gov/ij/> (accessed 3 September 2018). [↑](#footnote-ref-1)