**Title page**

**Computed tomographic identified mineralisation of the longitudinal odontoid ligament of the horse is associated with age and sex but not with the clinical sign of headshaking.**

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**Abstract**

Mineralisation of the longitudinal odontoid ligament has recently been identified in 3 horses undergoing computed tomographic (CT) examination, but the clinical relevance of this finding has yet to be investigated. The objective of this retrospective cross-sectional study was to investigate the relationship of this image finding to primary presenting complaint, age, breed, use and sex of the patient and determine any association to the clinical signs of head shaking, neck pain or restricted range of neck motion. CTImages of horses undergoing examination of the head and cranial cervical spine, for a variety of clinical reasons, were assessed for the presence of mineralisation within the longitudinal odontoid ligament. Clinical records were reviewed; presenting problem, signalment, clinical signs and final diagnoses were recorded and potential associations of presenting primary problem, signalment and individual clinical signs with mineralisation in the longitudinal odontoid ligament investigated, using univariable and multivariable ordinal logistic regression analysis. Final multivariable analysis confirmed significant associations of increasing severity of mineralisation with increasing age (*P* = 0.002) and being female (*P* = 0.038). There was no association of mineralisation of the longitudinal odontoid ligament with the syndrome of idiopathic head shaking or other clinical signs investigated. Veterinary Radiologists should be aware that mineralisation of the longitudinal odontoid ligament was not associated with specific primary complaint nor with specific clinical signs.

**Introduction:**

Mineralisation of the longitudinal odontoid ligament of the horse has recently been reported1. The study by Lawson et al. identified mineralisation of the longitudinal odontoid ligament during CT examination of three horses that presented for headshaking and poor performance. Idiopathic headshaking in horses is largely a diagnosis of exclusion with assumed or confirmed trigeminal neuralgia as the underlying causation2. Others have suggested that musculoskeletal pain may be contributing factor in some horses3. It is often a frustrating and debilitating condition to treat and as such the suggestion that mineralisation of the longitudinal odontoid ligament may be involved in this syndrome requires further investigation. Neck pain in horses is also a common and non-specific presenting problem and clinical finding. Imaging of the cervical spine in the horse has historically been difficult due to the large size of patients compared with humans and species encountered in small animal practice. Recent improvements in advanced imaging equipment have allowed imaging of this region in equines to be more widely available. As such there are image findings that the veterinary radiologist may encounter for which the significance of the lesion is not currently documented.

The clinical significance of mineralisation of the longitudinal odontoid ligament in the horse is currently undetermined. There are numerous reports of clinically significant mineralisation of the ligamentous structures of the occipitoatlantoaxial joints in humans4, 5 and a single report of a similar condition in a dog;6 The purpose of this retrospective cross-sectional study is to estimate the prevalence of mineralisation of the longitudinal odontoid ligament of horses and to determine any association with primary presenting problem, signalment or clinical signs.

The anatomy of the ligaments of the occipital atlantoaxial region differs in horses compared with other mammas.7, 8 The anatomy of this region in the horse, as determined by 3T MRI examination, has been reported in detail.9 In brief, the longitudinal odontoid ligament is a sole, robust, fan shaped, bi-lobed, ligament that extends from the cranial part of the dens of C2 and attaches cranially to the floor of the body of C1. In contrast, in dogs and humans the analogous apical ligaments extend through the body of C1 to insert primarily ventrolateral to the foramen magnum. Horses also lack the alar ligaments and transverse ligament of the dens seen in other species. Clayton et al.10  demonstrated high overall ranges of axial rotation and dorsoventral flexion of the cervical spine between C1-C2 in cadaver spines of foals and adult horses. They also showed that dorsoventral flexion at the C1-C2 location was significantly greater in foals than in adults. Considering these findings, the longitudinal odontoid ligament maybe of potential clinical interest in animals with clinical signs of neck pain or restricted range of motion of the neck, headshaking or poor performance.

We hypothesised that (a) there will be an increased prevalence of mineralisation of the longitudinal odontoid ligament in older horses (b) there will be no association between presence of mineralisation of the longitudinal odontoid ligament of the horse and breed, sex, height or use of the horse (c) that mineralisation of the longitudinal odontoid ligament will be associated with both the primary presenting complaint of idiopathic headshaking and neck pain and with the clinical signs of headshaking and neck pain.

**Methods:** This was a retrospective analytical cross-sectional study design. The images of all horses that had undergone a head or neck CT examination between February 2018 and August 2019 at the study hospital were reviewed. All horses in which the longitudinal odontoid ligament of the C1-C2 articulation could be viewed in entirety and for which full clinical records were available were chosen for inclusion in the study. Horses were excluded if the longitudinal odontoid ligaments could not be viewed in entirety from the cranial attachment on C1 to the caudal attachment on C2 . The study had prior approval by the University of Liverpool Ethics Review Committee, approval number VREC814

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The clinical records were reviewed by one of the authors,( an intern in equine studies),MRblinded to the results of the CT examination. The age, breed, use, sex, primary presenting problem and presence or absence of clinical signs of head shaking, neck pain (as evidenced by reduced range of cervical spine range of motion or resentment of palpation of the neck) or abnormal behaviour were recorded as a binary yes or no. The findings of clinical examination on presentation at the hospital were recorded. Horses were grouped into those that were referred for investigations of problems other than headshaking or neck pain and therefore hypothesised as being not associated with the presence of mineralisation the longitudinal odontoid ligament (Group 1) and horses referred for further investigations of suspected idiopathic headshaking, or neck pain as a primary compliant and in which mineralisation of the longitudinal odontoid ligament may be of concern.(Group 2).

The CT images were reviewed by a large animal resident of the ECVDI (AT) who was blinded to the clinical history and findings of the clinical examination of each case at the time of image viewing. Images were viewed on a computer monitor, using proprietary DICOM software(HOROSTM, GNU Lesser General Public License, Version 3.0, LGPL 3.0) in transverse views and as 3D multiplanar reconstructions. The longitudinal odontoid ligament was subjectively assessed on bone (WL 300, WW 1500) and soft tissue (WL 35, WW 350) windows according to size, shape, margination and attenuation. The presence or absence of mineralisation of the longitudinal odontoid ligament was recorded. Images were objectively analysed, using a region of interest (ROI) drawn around the full transverse sectional area of both lobes of the ligament at the cranial portion of the dens. The maximum (Max) and minimum (Min) Hounsfield unit (HU) and standard deviation (SD) values were recorded. Based on the combined subjective and objective analysis of images, mineralisation was further categorised as none (grade 0), mild (grade 1), moderate (grade 2) and marked (grade 3). The defining features for classification of mineralisation grade are summarised in **Table 1.**

Statistical analyses were carried out using the statistical software SPSS 25.0 (SPSS Inc, Chicago, Illinois, USA) and R (R version 3.2.0, The R Foundation for Statistical Computing) by an ECVDI boarded radiologist(T.W.M) experienced in statistical analysis. Independent variables were generated from signalment data, history and clinical examination. Variables examined were those related to the horse (breed, sex, age, height, use), history and clinical examination findings (presence or absence of clinical signs of head shaking, neck pain, restricted neck movement , abnormal behaviour and poor performance) and principle presenting complaint (i.e. Group 1 or 2).

Descriptive statistics were calculated for variables as required; continuous data were summarised as median values with interquartile ranges (IQR), and categorical data as frequencies including 95% confidence intervals (95% CI) if appropriate. Categorical variables with large numbers of categories and/or categories with small numbers of individuals were reviewed and, if necessary, categories were collapsed into larger groupings. The continuous variables of age and height were assessed with a test for departure from linear trend to determine whether an assumption of linear association was valid. Distribution of these continuous variables was assessed using the Kolmogorov-Smirnov test.

Potential associations between the categorical severity of longitudinal odontoid ligament mineralisation and the independent variables were examined using ordinal logistic regression, with mineralisation severity (zero, mild, moderate, severe) as the ordinal outcome. Variables demonstrating some association with mineralisation severity on univariable analysis (*P*-value <0.2) were selected for inclusion into a multivariable model in accordance with accepted statistical procedure11. The final multivariable model was built using a manual backwards stepwise procedure with retention of variables with Wald *P*-values < 0.05.

**Results**

125 horses underwent CT examination of the head at the study institution during the study period. The majority of CT examinations were performed with the patients restrained with standing sedation using a combination of intravenous acepromazine, (0.03mg/kg), morphine (0.1mg/kg) and romifidine (0.06-0.08mg/kg). A small number of patients, due to lack of patient compliance or due to a request to include all of the cervical spine, underwent general anaesthesia and CT examination was then performed with the patient positioned in right lateral recumbency. General anaesthesia was induced with ketamine (2.7mg/kg i.v) and diazepam (0.07mg/kg i.v). Following induction, general anaesthesia was maintained with sevoflurane with an end-tidal anaesthetic agent concentration of between 3.1 – 3.2%. Included horses were presented for CT examination for a variety of reasons.

Images were obtainedusing a 16 slice, 90cm bore, multidector, CT scanner(Canon Aquillion, Canon Medical Systems Ltd. Crawley, UK) XXXXXXXXXXXX. The head and neck of the patient were supported on a custom made, radiolucent carbon fibre table(XXXXXXXXXXXX). Scanning parameters varied slightly between patients; most images were acquired using 16 row x 1.0mm detector width, 120 KVp and 300 mAs, 550mm field of view, 1.0 or 0.5mm slice thickness, tube rotation time 0.75secs and gantry pitch 0.688. All images were reconstructed using a field of view of 400mm and a soft-tissue algorithm and bone algorithm.

96 horses met the inclusion criteria for the study. 29 horses were excluded due the ligaments of C1-C2 not being visible in entirety on multiplanar images. Full clinical records and results of clinical examinations were available for all horses. The clinical examination for all horses on arrival at the hospital followed a standardised protocol and was performed by either a ECVS certified equine surgeon, ECEIM equine internal medicine or ACVSMR equine sports medicine clinician or resident, overseeing the case on admission. If, after the initial examination, neurological deficits were suspected then further proprioceptive evaluation was performed. If dental disease was suspected, or reported, oral examination with a full mouth speculum and dental mirror was also performed. For horses reported to headshake or exhibit neck pain additional dynamic evaluation was undertaken.

Of the 96 horses included in the study 37/96 (38.5%) were female 59/96 (61.5%) were male Ages ranged from 1yr to 24 yrs, (median 10, range 23).There were 33/96 (34.4%) ponies, 22/96 (22.9%) Warmbloods (WB), 18/96 (18.75%) Thoroughbred (TB) or TB-crosses; 7/96 (7.29%) Draft horses and 16/96 (16.6%) other breeds (11 Irish sports horse, 3 Friesian, 2 Arabian). Grouping of horses according to primary presenting problem is summarised in **Table 2.**

Signalment of all horses, presenting problem and pertinent clinical findings are summarized in Appendix 1.

On subjective image evaluation the majority of horses in the study 66/96 (68.8%; 95%CI 59.4-78.0%) presented a symmetrically shaped, bilobed ligament, which was well demarcated and clearly marginated, with a heterogeneous attenuation, in a regularly distributed, subtle striped pattern (Fig 1).

Following subjective and objective ROI analysis, 25/96 (26%; 95%CI 17.3-34.8%)horses were identified as having some degree of mineralisation in the longitudinal odontoid ligament; 8/25 (32%) were categorised as marked (grade 3), 5/25 (20%) as moderate (grade 2), 12/25 (48%) were classified as mild (Grade 1). Examples of each grade of mineralisation are given in Figure 2.

In five horses without mineralisation, other abnormal image findings of the longitudinal odontoid ligament and its caudal attachment site on C2 were recorded. Three of these five horses had discrete, focal, osseous cyst-like lesions of the cranial portion of the dens at the site of the caudal attachments of the longitudinal odontoid ligament. Four of these same five horses also had subjective enlargement of one or both lobes of the ligament, poor definition of the borders of the ligament or focal areas of hypoattenuation. One of these five horses had focal areas of hypoattenuation, enlargement and poor definition of the borders of each lobe of the ligament without any bony changes (Fig 3).

Out of the overall number of horses with mineralisation in the longitudinal odontoid ligament (n=25); a greater proportion of horses were female (15:25) compared with males (10:25), despite an overall lower number of female horses in the study compared with males (37:59)

Horses without mineralisation (n=71) had a lower mean age (mean 9.61, min 1, max 20.33, range 19.33) than horses with mineralisation of any grade (n=22) (mean 12.95, min 3.42 max 24, range 20.58). Those with mild mineralisation, grade 1, (n=12) had slightly greater mean age (mean 9.88, min 3.5, max 18.5, range 15) compared to the horses without mineralisation, followed by those with moderate mineralisation, grade 2 (n = 5) (mean 14.18, Max 22, Min 9.08, Range 12.92). The group identified as having marked mineralisation, grade 3 (n=8), had the highest mean age of the groups of horses (mean 16.79, min 13, max 24, range 11) (**see Fig 4**.)

Some degree of mineralisation was found in the longitudinal odontoid ligament of12/33 (36.4%) ponies; 4/11 (36.4%) ISH; 6/18 (33.3%) TB(X); 3/22 (13.6%) WB; Mineralisation was not identified in the longitudinal odontoid ligament of any of the Draft, Friesian or Arabian horses.

Out of 25 horses identified with some degree of mineralisation in the longitudinal odontoid ligament; 18/25 (72%) horses were in Group**1** (dental =7, neurological = 4, nasal discharge =4, epistaxis =1, acute trauma =2) and 7/25 (28%) horses were in Group 2 (headshaking 5, neck pain 1, head shaking and neck pain 1).

The results of the subjective and objective CT image evaluations of the longitudinal odontoid ligament and full imaging findings, along with final diagnoses, are summarised in **Supplementary data 1**.

4/8 (50%) horses that hadmarked mineralisation identified in the longitudinal odontoid ligament were classified in group 1 in relation to presenting clinical signs (1 dental, 1 epistaxis, 1 nasal discharge, 1 neurological) and 4/8 (50%) horses were in group 2 (3 headshakers, 1 neck pain)

4/5 (80%) horses with moderate mineralisation were in group 1 (1 acute trauma, 2 nasal discharge, 1 neurological) and 1/5 (20%) horses were in group 2 (neck pain and head shaking combined)

10/12 (83.3%) horses with mild mineralisation were in group 1 (6 dental, 2 neurological, 1 acute trauma, 1 nasal discharge) and 2/12 (16.7) horses were in group 2 (2 headshaking).

Age was the only continuous variable to demonstrate a normal distribution, but neither age nor the other continuous variable of height showed a significantly non-linear relationship with the outcome considered and so both were considered for inclusion in the ordinal logistic regression analysis.Univariable analysisof the effect of group of horses, according to primary presenting complaint, showed no evidence of association with the mineralisation of the longitudinal odontoid ligament (P=0.996) and so horse group was not entered into the final multivariable analysis model. Univariable analysis of signalment and individual clinical signs; however, showed some potential evidence of association (P < 0.2) with the severity of longitudinal odontoid ligament mineralisation and the variables: age (P = 0.001), height (P = 0.16), gender (P = 0.019), presence of neck pain (P = 0.026), restricted movement (P = 0.11) and poor performance (P = 0.16). Following final multivariable analysis, significant associations with increasing severity of mineralisation were confirmed for only increasing age (*P* = 0.002) and being female (*P* = 0.038).

**Discussion**

In the current study mineralisation in the longitudinal odontoid ligament was a reasonably common finding on CT examination of the equine neck, identified in nearly a quarter of horses that met the defined inclusion criteria of this study. Perhaps unsurprisingly mineralisation was also more commonly found in older horses and ponies, however; the finding of the effect of sex and association with being female was not expected. The study also did not support the research hypothesis that mineralisation would be associated with head shaking or neck pain.

There are very limited number of reports of pathology of the longitudinal odontoid ligament in the horse. In contrast, mineralisation of the ligaments of the occipitoatlantoaxial junction is a well-recognised clinical entity in man of suspected multifactorial aetiology, including trauma, systemic disease and local inflammatory processes.4.5 A recent report1 described the finding of mineralisation of the longitudinal odontoid ligament of 3 horses presented for clinical signs of head shaking and/or neck pain; however, the only other reports of pathology of these ligaments in equines that exist in the literature relate to subluxation of this area and presumed complete disruption of these ligaments12, 13, 14 or malformations of the occipital-atlantoaxial region.15 It must be noted that in these case reports terminology of the anatomy of the ligaments is confusing. A detailed review of the anatomy of the ligaments, as viewed using high field MRI is given by Gutiérrez-Crespo et al.9 and is the anatomical terminology adopted by the authors of this paper.

. Figures relating to overall prevalence of mineralisation of equine ligaments are lacking. A recent study evaluating the presence or absence of mineralisation in the musculoskeletal system of the horse (as detected by ultrasonographic examination), identified mineralisation most commonly in the DDFT and the suspensory ligament branches with an estimated 10% and 7% of DDFT and suspensory ligament branch injuries respectively demonstrating this feature. Mineralisation was also identified in some horses without any additional evidence of tendinopathy of the DDFT, although it was noted that mineralisation preceded the development of hypoechoic foci in some lame horses. In contrast to the majority of ligaments of the appendicular skeleton, the longitudinal odontoid ligament cannot be completely assessed with ultrasound due to the lack of a suitable acoustic window at this location. The findings of our study suggest that mineralisation of the longitudinal odontoid ligament may be more common than in other equine ligaments. Guteierrez-crespo et al 9 suggested that the presence of connective tissue between the longitudinal odontoid ligament fibrils, with moderate to high signal intensity on T2-weighted MRI images, may be due to a significant fibrocartilaginous portion of the ligament reflecting the strong forces this region undergoes. A degenerative process, from accumulated microdamage in response to high biomechanical forces, in this area may explain the relatively high prevalence of mineralisation identified within this ligament on CT examination. Further correlation of CT image findings with histopathological findings would be necessary to identify the underlying macroscopic and microscopic changes.

Subjective assessment of the longitudinal odontoid ligament on CT images also identified a small minority of cases where focal or diffuse areas of hypoattenuation or marked asymmetry of the two lobes of the ligament were present without mineralisation. In addition, some horses had discrete osseous cyst like lesions evident at the caudal attachment of the ligament on the dens. The authors suggest that it is possible that such changes represent a spectrum of findings relating to a previously unrecognised syndrome of desmitis of this ligament. Of particular interest was the youngest horse in the study (12 months) that was found to have marked asymmetry of the ligament lobes, ligament enlargement and multiple hypoattenuating foci evident. This horse presented for ongoing complications following head trauma and frontal bone fracture, sustained as a day-old foal, with subsequent chronic sinusitis. In man, Grisel’s syndrome, a condition mainly identified in children, results in non-traumatic, atlantoaxial rotatory subluxation typically following infections of the head and neck or following surgical procedures of this region. The presumed aetiopathogenesis is increased inflammation of the region due to communication of the pharnyngovertebral veins and subsequent development of ligament laxity 17, 18 There was no suggestion of instability of the C1-C2 region in this horse; however, the CT image findings were interesting given the history of head trauma and chronic sinusitis and were consistent with the CT appearance of histologically confirmed chronic desmitis reported in the suspensory ligament branches of a horse. 19 Contrast enhanced CT examination has also been shown to be more sensitive and specific than evaluation of plain CT images alone in identifying lesions in equine tendons and ligaments  20, 21 and may be a consideration for further studies seeking evidence of pathology of the longitudinal odontoid ligament.

The significant association of increasing age with prevalence and degree of mineralisation of the longitudinal odontoid ligament was not unexpected and is in agreement with our first hypothesis, although it is not possible to determine from this study if this is a normal adaptive or pathological age-related process. Contrary to our second hypothesis we identified a significant association of being female with prevalence and degree of mineralisation of the longitudinal odontoid ligament. This was a surprising finding and is not readily explained. No information was available regarding whether the female horses had been bred from, which may be of relevance in further investigating ligament pathologies, possibly due to a hormonal link of pregnancy/parturition. Previous studies have investigated the association of sex with risk of injury of the superficial digital flexor tendon, deep digital flexor tendon and of the suspensory ligament in horses;22 with no effect of gender found, whereas Kasashima et al.23  identified a higher risk of superficial digital flexor tendonitis and suspensory ligament desmitis in males. There is little evidence in the literature for a strong overall effect of either sex on ligament pathology in general in the horse.

Thomson *et al.* 3 discussed the challenges in differentiating between musculoskeletal pain and trigeminal-mediated headshaking. Our study refuted our third hypotheses, in that mineralisation of the longitudinal odontoid ligament was not associated at any level with the clinical signs of headshaking.

The study has a number of recognised limitations. There is potential forpopulation selection bias due to the included hospital population.There are arelatively small number of horses in each group of presenting clinical signs, particularly in the group presented for headshaking behaviour, reducing the statistical power of the study. Additionally, images were viewed and reported by a single observer and there was no test of repeatability of image grading.

Further studies including histopathological analysis of the longitudinal odontoid ligament and comparison to image findings are warranted. None of the horses in the present study were subjected to euthanasia and therefore histopathological analysis was not available for any of the included cases.

**Conclusions**

Findings from the current study indicated that veterinary radiologists should be aware that mineralisation within the longitudinal odontoid ligament is a reasonably common finding in horses undergoing CT examination of the cranial cervical region. Mineralisation of the longitudinal odontoid ligament in horses may be considered age-related and is more likely to be evident in female horses. There is no link to the syndrome of idiopathic head shaking. The presence of marked mineralisation in younger animals is unusual and may be a reflection of desmitis of this ligament. Further investigations are warranted to fully investigate the clinical relevance of CT image findings in this structure.

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**List of author contributions**

Category 1

1. Conception and design: Talbot, Maddox
2. Analysis and interpretation of data: Talbot, Maddox, Rodrigues XX

Category 2

1. Drafting the article: Talbot, Rodrigues, Maddox XX
2. Revising the article for intellectual content: Talbot, Rodrigues Maddox XXXXXX

Category 3

1. Final approval of the completed article: Talbot, Rodrigues, Maddox XXX

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**Table 1. Four point scoring scale used for categorising CT image findings of mineralisation within the longitudinal odontoid ligament**

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| --- |
| Grade Description Definition |
| 0 Normal No areas of mineral attenuation identified and region  of interest (ROI) analysis Max HU < 300  1 Mild Small focal discrete areas of mineral attenuation in  one or both lobes of the ligament, no larger than  2mm x 2mm in size. ROI analysis Max HU > 3002 Moderate Focal, discrete areas of mineralised attenuation in  One or both lobes of the ligament where some or all  of the area of mineralisation are greater than 2mm x  2mm in size, but no greater than 3mm in any  direction. There is no change in size  of either ligament lobe. ROI analysis Max HU > 3003 Marked Focal, larger (>3mm in at least one direction ) areas  of mineral attenuation in one or both lobes of the  ligament and in which there is concurrent lobe  enlargement or lobe asymmetry or in which the  margins of the ligament are distorted.  ROI analysis Max HU > 300 |

**Table 2: Summary of grouping of horses according to primary presenting problem**

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| --- |
| Primary presenting problem Number of horses  |
| Group 1 (*n=71*) Dental disease 21 Epistaxis 8 Upper respiratory tract 5 Acute trauma 5 Neurological deficits 17 Nasal discharge 14 Aural infection 1 |

**Table 3. Results of multivariable ordinal logistic regression for variables associated with the severity of mineralisation of the longitudinal odontoid ligament in 96 horses. *P*-values are from the Wald chi-square test.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Category** | **Odds ratio (95% CI)** | ***P*-value** |
| **Age (months)** |  | 1.01 (1.00-1.02) | 0.002\* |
| **Sex** | Male | Ref | - |
|  | Female | 2.78 (1.06-7.3) | 0.038\* |

95% CI = 95% confidence interval

**Figure Legends**

**Fig 1. A-F.** Transverse (A, B), dorsal (C, D) and parasagittal plane (E, F) CT images in a modified bone WL 300 WW 1500 (A, C, E) and soft tissue, WL 35, WW 350 (B, D, F) window, at the level of the longitudinal odontoid ligament. Parasagittal images are centred over the left lobe of the odontoid ligament. Transverse images left is on the right and top is dorsal. Dorsal plane images, left is on the right and top is rostral, parasagittal plane images, rostral is on the left and dorsal is top. The most commonly encountered appearance of the longitudinal t odontoid ligament was that of a bi-lobed, symmetrical and well marginated structure with slightly striped pattern of soft tissue attenuation (blue arrow heads). The caudal attachments of the ligament are on the odontoid process (blue star) of the atlas (C2) and the cranial attachments are on the body of the axis (C1).

**Fig 2.** Transverse (A, C, E) and parasagittal plane (B, D, F) CT images in a modified bone window WL 300, WW 1500, demonstrating grade 1 (A, B), grade 2 (C, D) and grade 3 (E, F) mineralisation of the longitudinal odontoid ligament.

Transverse plane images are at the cranial level of the odontoid process of C2, parasagittal images are at the level of the left lobe of the longitudinal odontoid ligament. Transverse images left is on the right and top is dorsal. Parasagittal plane images, rostral is on the left and dorsal is top.

Example of grade 1 - There are two, focal, areas of mineralisation of less than 2mm in any direction, seen in the left lobe of the longitudinal odontoid ligament (red arrows). There is no enlargement of the lobes of the ligament and the margins of the ligament are clearly defined (A, B). Example of grade 2 – there are multiple focal areas of mineralisation within the longitudinal odontoid ligament that exceed 2mm in length in at least one direction (blue arrow heads), there is minimal lobe enlargement evident (C, D). Example of grade 3 – there are multiple, focal areas of mineralisation within the ligament some of which exceed 2mm in length in at least one direction (blue arrows). There is enlargement of both of the ligament lobes and loss of the definition between the dorsal border of the ligament and the ventral border of the spinal cord (E, F).

**Fig. 3** Transverse (A, B), parasagittal (C, D) and dorsal plane (E, F) CT images in a modified bone WL 350, WW 1500 (A, C ,E) and soft tissue WL 35, WW 350 (B, D, F) window demonstrating examples of variations in appearance of the longitudinal odontoid ligament and its attachment encountered during the study**.** Parasagittal images are centred over the left lobe of the odontoid ligament. Transverse images left is on the right and top is dorsal. Dorsal plane images, left is on the right and top is rostral, parasagittal plane images, rostral is on the left and dorsal is top.

There is a rounded osseous cyst like lesion (blue arrows) in the left hand side of the odontoid process at the caudal attachment of the left lobe of the longitudinal odontoid ligament (A, C, E). There is a focal area of hypoattenuation in the centre of the left (blue arrow heads) and right lobe of the longitudinal odontoid ligament (B, D F).

**Figure 4.** Box plot showing the ages of 96 horses within the four grades (0-3) of mineralisation of the longitudinal odontoid ligament. Each box represents the interquartile range, the horizontal line within each box represents the median, the whiskers represent the range (excluding outliers), and circles represent outliers.