# **ORIGINAL ARTICLE**



# Congenital variants of the ventral laminae of the sixth and seventh cervical vertebrae are not associated with clinical signs or other radiological abnormalities of the cervicothoracic region in Warmblood horses

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#### **Abstract**

**Background:** There is controversy about the clinical relevance of congenital variants of the ventral laminae of the sixth (C6) and seventh (C7) cervical vertebrae and their relationship with other radiological abnormalities.

**Objectives:** To document the prevalence of congenital variants of C6 and C7 and that of other radiological abnormalities from C6 to the second thoracic vertebra (T2). **Study design:** Cross-sectional.

Methods: The study included Warmblood horses ≥3 years of age undergoing clinical assessment at two referral institutions: 127 control horses and 96 cases (neurologic, neck pain or stiffness, or neck-related forelimb lameness). All horses underwent a standardised orthopaedic and neurologic examination. Lateral–lateral and lateral  $45^{\circ}-55^{\circ}$  ventral–lateral dorsal (left to right and right to left) radiographic views of C5 to T2 were acquired and assessed blinded to the horse's clinical category using a predetermined grading system.

**Results:** The ventral profile of C7 was abnormal in 54 horses (24.2%). Cases were less likely to have congenital variants than control horses, p = 0.0002, relative risk (RR): 0.63 (95% confidence intervals [CIs]: 0.4, 1.0). There was no association between the presence of a congenital variant of C7 and the presence of modelling of the articular processes (APs) of C6-C7, C7-T1 or T1-T2. Cases were more likely to have severe modelling of the APs at C6-C7, p = 0.01, RR: 1.94, CI: 1.1, 3.5 and C7-T1, p = 0.04, RR: 1.97, CI: 1.2, 3.2 compared with control horses.

**Main limitations:** Radiographs were read by one assessor independently at each institution.

**Conclusions:** There was no association between the presence of congenital variants of C7 and any other radiological findings. Congenital variants occurred less

Sue Dyson and Monica Aleman contributed equally to this study.

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frequently in cases compared with control horses. There was no association between the presence or absence of a congenital variant and the type of case.

#### KEYWORDS

ataxia, forelimb lameness, horse, neck stiffness, osteoarthritis, radiography

#### 1 | INTRODUCTION

Clinical signs such as neck pain, neck stiffness, forelimb lameness and ataxia have been associated with radiological abnormalities of the caudal cervical vertebrae. Congenital variants of the sixth (C6) and seventh (C7) cervical vertebrae have been described, with transposition of one or both ventral laminae of the transverse processes (subsequently referred to as the ventral laminae) from C6 to C7. However, there are limited detailed descriptions of the association of these variants with radiological abnormalities of the cervicothoracic region and there is conflicting evidence about their clinical relevance.

It is recognised that radiological evidence of osteoarthritis (OA) of the caudal cervical articular process joints (APJs) may be present in showjumping horses performing at a high level without associated clinical signs.<sup>22</sup> However, in another study, the prevalence of radiological abnormalities of the APJs of C6–C7 or the APJs of C7 and the first thoracic vertebra (T1) graded ≥2/4, in a mixed group of low-level performance horses, was low.<sup>23</sup> There are other studies which have compared the presence of radiological evidence of modelling of the articular processes (APs) of the caudal cervical vertebrae and clinical signs with conflicting results.<sup>7,10,17,24</sup> Most studies have not included radiological assessment of the cranial thoracic vertebrae. In those studies which have related clinical features to radiological abnormalities there has not been a standardised method of clinical assessment of cases and control horses.<sup>7,10,13,17,24</sup>

Recent studies investigating computed tomographic assessment of the cervical vertebrae have indicated that enlargement (modelling) of APs can be present without signs consistent with OA, such as alterations in subchondral bone opacity and joint space width.<sup>25,26</sup> It is unclear to what extent modelling of the APs is a consequence of biomechanical forces acting on the APs versus reflecting the presence of OA.

The aims of this study were to document the prevalence of congenital variants of C6 and C7 and the prevalence of modelling of the APs of C6 to the second thoracic (T2) vertebra, spondylolisthesis (subluxation) of C6 to T2, or modelling of the APs and subluxation, or disease of the intervertebral symphysis<sup>27</sup> and adjacent vertebral bodies (discospondylosis) in horses with and without congenital variants of C6 and C7. An additional aim was to determine whether congenital variants occurred more commonly in horses with neck pain or stiffness, forelimb lameness attributed to nerve root compression, or neurological abnormalities consistent with a compressive lesion of the cervicothoracic spinal cord compared with control horses, all examined using a standardised protocol. It was hypothesised that there would be a higher prevalence of (1) congenital variants of C6 and C7 in cases compared with control horses; (2) modelling of the APs of C6–C7 and

C7-T1 in horses with congenital variants of C6 and C7 compared with horses without congenital variants; and (3) modelling of the APs of C6-C7 and C7-T1 in cases compared with control horses. In addition, it was hypothesised that the severity of modelling of the APs of C6-C7 and C7-T1 would be greater in cases compared with control horses.

# 2 | MATERIALS AND METHODS

# 2.1 | Clinical assessment and selection criteria of horses

A cross-sectional study of all Warmblood horses aged ≥3 years undergoing comprehensive clinical investigation at the Centre for Equine Studies, Animal Health Trust, UK (AHT) and the William R. Pritchard Veterinary Medical Teaching Hospital from the University of California Davis, USA (UCD) was performed. All horses underwent a standardised clinical assessment (Text S1) according to predefined criteria agreed upon by two authors (M.A. and S.J.D.) based on previous publications<sup>1-4</sup> and clinical experience. This included systematic visual examination (posture, muscle development, sweating) and palpation (muscle tone, hypoaesthesia, hyperaesthesia, allodynia, pain, cervical range of motion) at rest. During dynamic examination, horses were evaluated moving in hand on a firm surface at walk and trot, turning in small circles at walk, lungeing (when safe to do so) on soft and firm surfaces and ridden exercise, when appropriate, with particular reference to signs of musculoskeletal or neurological dysfunction. All clinical examinations were performed by two of the authors. Additional assessments and ancillary tests were made at the clinicians' discretion based on the history and clinical observations. Cases were classified as neurologic (general proprioceptive [spinal] ataxia), lame with neckrelated forelimb lameness (not responsive to perineural anaesthesia including median and ulnar nerves, not responsive to intra-articular anaesthesia of the humeroradial or scapulohumeral joints and no detectable radiological or ultrasonographic abnormality of the shoulder, elbow, first ribs and sternal regions), and those with neck pain and/or stiffness. Control horses were those with gait abnormalities that were abolished by diagnostic anaesthesia or horses undergoing pre-purchase examinations without any clinical signs.

# 2.2 | Radiography and radiological interpretation

Radiographic examination of the caudal cervical (C5-C7) and cranial thoracic (T1-T2) vertebrae was performed, with the acquisition of

computed (AHT) or digital (UCD) lateral-lateral (left to right or right to left) images of C5-T2 and left lateral 45°-55° ventral-right lateral dorsal oblique and right lateral 45°-55° ventral-left lateral dorsal oblique images of C5-C7 in all control horses and cases.<sup>28</sup> In addition laterallateral images were acquired from the occiput to C4 in all cases. Exposure factors were determined by patient size. A predetermined radiological grading system was developed (Texts S2 and S3) to describe the morphology of the vertebral bodies of C6 and C7, the presence and severity of modelling (alteration in shape and size) of the APs from C6 to T2, ± alterations in radiopacity, the size of the intervertebral foramina, the alignment of adjacent vertebrae, the uniformity of width and opacity of the intervertebral symphyses and alterations in shape and/or opacity of adjacent bones (discospondylosis). The presence of asymmetry in the size of the APs was determined from a combination of the lateral-lateral and both oblique radiographs. Before data collection radiological interpretation of 10 sets of radiographs was performed by a Diplomate of the American College of Veterinary Radiology and an Associate of the European College of Veterinary Diagnostic Imaging to reach a consensus in interpretation of the grading system. Thereafter, the radiographs were interpreted independently, without knowledge of the clinical status of each horse, and the results were recorded in a purpose-designed Microsoft Excel spreadsheet (Version 2010).

# 2.3 | Data analysis

All data analyses were performed using SAS® 9.4 (SAS Institute Inc). with significance set at p < 0.05. Based on an estimate of the presence of congenital variants in 29% of cases and 17% of control horses, for a study with 80% power at a significance level of p < 0.05, an a priori sample size calculation determined that 32 subjects per group were required. The distribution of continuous variables (horse age, bodyweight and height) was tested for normality using the Shapiro-Wilk test, in combination with visual assessment of histograms, with overlaid kernel density plots. Results were expressed as means ± standard deviations (SDs) and range. Categorical variables were summarised as proportions and expressed as percentages. Denominators for controls and cases were 127 and 96, respectively, except when stated otherwise. Relationships between categorical variables were assessed using the Chi-square  $(\chi^2)$  or Fisher's exact test when observed counts in any comparison group were<5. Where appropriate, relative risks (RRs) with 95% confidence intervals (CIs) were calculated.

#### 3 | RESULTS

# 3.1 | Study population

The population comprised 223 Warmblood horses, including 127 control horses and 96 cases. Forty-seven (49.0%) of the cases were classified as neurologic, 31 (32.3%) had neck-related forelimb lameness, and 18 (18.8%) had neck stiffness and/or pain. There were 148 geldings, 6 stallions and 69 mares. Work discipline included dressage

( $n=99,\ 44.4\%$ ), showjumping ( $n=57,\ 25.6\%$ ), eventing ( $n=48,\ 21.5\%$ ) and general purpose, including unaffiliated competition ( $n=19,\ 8.5\%$ ). Control horses were younger (mean 8.5 years SD  $\pm$  3.0, range: 3–19) than case horses (mean 9.6 years SD  $\pm$  4.0, range: 3–22), p=0.03. Mean bodyweight (n=187) was 584 kg (SD  $\pm$  59, range: 372–700) and mean height (n=159) was 167 cm (SD  $\pm$  5, range: 145–180). There was no significant difference between the bodyweights and heights of control horses and cases. The prevalence of all radiological abnormalities and their relationship with the presence of congenital variants of C6 and C7 are documented in Table S1. The prevalence of variants of C6 and C7 and other radiological abnormalities stratified by control horses and cases is summarised in Table S2.

#### 3.2 | Ventral laminae of C6 and C7

One or both ventral laminae of C6 were absent in 18 (8.1%) and 35 (15.7%) horses, respectively (Figure 1A-D). The ventral profile of C7 was abnormal in 54 horses (24.2%) (Figure 1B-D); one horse in which C6 appeared normal had a vestigial caudal ventral lamina on C7 (Figure 1D). In 51 horses, the ventral lamina(e) extended from the caudal aspect of the vertebral body more than half the length of the vertebral body (Figure 2A), in one of which there was an additional ventral lamina which extended further ventrally (Figure 1C). In two horses, the ventral lamina(e) extended from the caudal aspect of the vertebral body less than half the length of the vertebral body. A congenital variant of C7 was present in 38 of 127 (29.2%) control horses and 16 of 96 (16.7%) cases; cases were less likely to have congenital variants than control horses, p = 0.02, RR: 0.63 (CI: 0.4, 0.97). Transposition of both ventral laminae was less likely in cases (10, 10.4%) compared with control horses (25, 19.7%), p = 0.04, RR: 0.61 (CI: 0.35, 1.04). Cases were less likely to have a ventral lamina on C7 extending from caudal to more than half the length of the vertebral body (16, 16.7%) than control horses (35, 27.6%), p = 0.05, RR: 0.66 (CI: 0.43, 1.03).

#### 3.3 | Modelling of the APs

There was no difference in the prevalence of modelling of the APs of C6–C7 in horses with (47/54, 87.0%) or without (155/169, 91.7%) congenital variants of C7 (Figures 1C and 2A–E). There was no difference in the prevalence of modelling of the APs in cases (88, 91.7%) compared with control horses (114, 98.7%). There was no difference between the degree of modelling of the APs at C6–C7 (mild, moderate or severe) and the presence or absence of a congenital variant of C7 (Table 1). However, cases were more likely to have severe modelling compared with control horses, p=0.01, RR: 1.94 (CI: 1.06, 3.54) (Table 2). Asymmetry in the size of the APs was determined from a combination of the lateral–lateral and both oblique radiographs, and data were available for C6–C7 for 217 horses, 125 control horses and 92 cases. There was no significant difference in the presence of

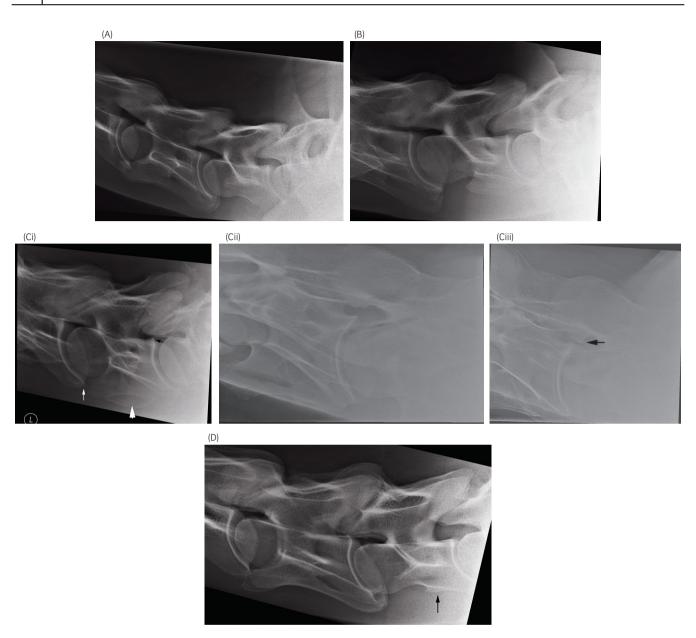


FIGURE 1 (A) Lateral-lateral radiographic image of the fifth cervical (C5) to second thoracic vertebrae of a control horse of 5 years of age. Cranial is to the left. There is a normal ventral profile of the vertebral bodies of both the sixth (C6) and seventh (C7) cervical vertebrae. The articular processes of C5-C6, C7-T1 and T1-T2 are normal. There is mild modelling of the articular processes of C6-C7. The intervertebral foramina are all normal in size. The vertebrae are normally aligned. (B) Lateral-lateral radiographic image of the sixth cervical (C6) to first thoracic (T1) vertebrae of a control horse. Cranial is to the left. Both ventral laminae of C6 are transposed to the vertebral body of the seventh cervical (C7) vertebra and extend slightly less than half the length of the vertebral body. There is mild modelling of the dorsocaudal aspect of the vertebral fossa of C7. The C7-T1 articular processes are moderately enlarged, with a narrowing of the intervertebral foramen. (C) (i) Lateral-lateral radiographic image of the sixth cervical (C6) to first thoracic (T1) vertebrae of a case with neck pain/stiffness. Cranial is to the left. Both ventral laminae of C6 are transposed to the vertebral body of the seventh cervical (C7) vertebra; there is an additional lamina which extends further distally (arrowhead). The ventral processes extend more than half the length of the vertebral body. The articular processes of C6-C7 and C7-T1 are asymmetrically moderately enlarged (modelled). There is a narrowing of the intervertebral foramina at C6-C7 and C7-T1. The head of the vertebral body of C7 is slightly displaced ventrally. There is slight modelling of the ventrocaudal aspect of the vertebral fossa of C6 (spondylosis deformans) (white arrow). The head of the vertebral body of T1 is slightly displaced dorsally. There is mild modelling of the dorsocaudal aspect of the vertebral fossa of C7 (black arrow). (C) (ii) Left 45° ventral-right dorsal oblique image of the seventh cervical and first thoracic vertebrae of the same horse as in Ci and Ciii. Cranial is to the left; left is to the top. The left articular processes (at the top of the image) are smaller than the right. Compare with Ciii. (C) (iii) Right 45° ventral-left dorsal oblique image of the seventh cervical and first thoracic vertebrae of the same horse as in Ci and Cii. Cranial is to the left; the right is to the top. The right articular processes are larger than the left (compare with Cii), and there is an osseous spur (arrow) projecting into the intervertebral foramen. (D) Lateral-lateral radiographic image of the fifth cervical (C5) to first thoracic (T1) vertebrae of a control horse. Cranial is to the left. There is a vestigial ventral lamina on the caudoventral aspect of the vertebral body of C7 (arrow). There is moderate enlargement of the articular processes of C6-C7. There is slight ventral displacement of the cranial aspect of the vertebral body of C7 and subtle modelling of the ventrocaudal aspect of the vertebral fossa of C6.

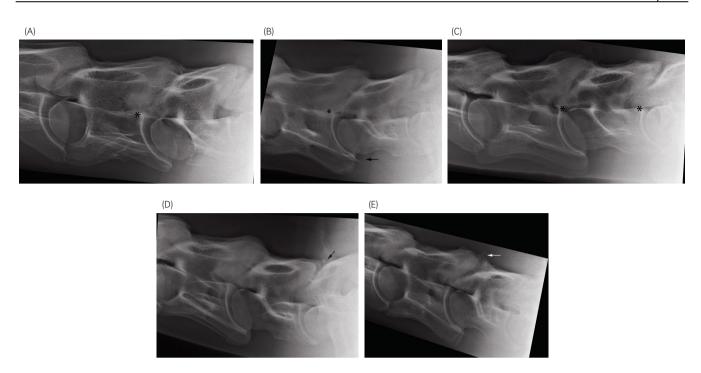


FIGURE 2 (A) Lateral-lateral radiographic image of the fifth cervical (C5) to first thoracic (T1) vertebrae of a control horse. Cranial is to the left. Both ventral laminae are transposed to C7 and extend more than half the length of the vertebral body. There is severe modelling of the articular processes of C6 and C7, with ventral buttressing (asterisk) and obscurement of the intervertebral foramen. (B) Lateral-lateral radiographic image of the sixth cervical (C6) to first thoracic (T1) vertebrae of a case with ataxia and proprioceptive deficits. Cranial is to the left. There is severe modelling of the articular processes of C6-C7, with ventral buttressing (asterisk) and narrowing of the intervertebral foramen. There is a large spur on the ventrocaudal aspect of the vertebral fossa of C6 (arrow). The C6-C7 intervertebral disk space is slightly wider dorsally compared with ventrally, with slight ventral displacement of the cranial aspect of the vertebral body of C7. The cranial aspect of the vertebral body of T1 is slightly displaced dorsally. There is a mild enlargement of one of the articular processes at C7-T1. Note also the well demarcated circular radiolucency in the vertebral body of C6. (C) Lateral-lateral radiographic image of the fifth cervical (C5) to the first thoracic (T1) vertebrae of a case with neck-related forelimb lameness. Cranial is to the left. There is moderate modelling of the articular processes of C6-C7 and C7-T1 with ventral buttressing (asterisk) and the intervertebral foramina are obscured. (D) Lateral-lateral radiographic image of the sixth cervical (C6) to the first thoracic (T1) vertebrae of a control horse. Cranial is to the left. The caudoventral profile of the vertebral body of the seventh cervical vertebra (C7) is slightly angular in appearance. There is moderate modelling of the articular processes of both C6-C7 and C7-T1. The intervertebral foramen at C6-C7 is narrowed; the intervertebral foramen at C7-T1 is obscured. There is a small, well-defined mineralised opacity dorsal to the articular process joints of C7-T1 (arrow). There is a mild dorsocaudal epiphyseal 'flare' of the vertebral body of C6. (E) Lateral-lateral radiographic image of the fifth cervical (C5) to the first thoracic (T1) vertebrae of a control horse. The cranial is to the left. There is moderate enlargement of the articular processes of C6-C7 and narrowing of the intervertebral foramen. There is a mineralised opacity dorsal to the articular process joints of C6-C7 (arrow). The articular processes of C7-T1 are mildly enlarged. There is a mild epiphyseal flare of the dorsocaudal aspect of the vertebral body of C6.

asymmetry in horses, with 6 of 53 (11.3%) a congenital variant of C7 compared with no congenital variant 21 of 164 (12.8%), p=0.78. However, cases (26/92, 28.3%) were more likely to have asymmetry compared with control horses (1/125, 0.8%), p<0.001, RR: 2.77 (CI: 2.25, 3.41).

There was no significant difference between the prevalence of modelling of the APs of C7-T1 in horses with (33/54, 61.1%) or without (80/169, 47.3%) congenital variants of C7. There was no difference between the degree of modelling of the APs at C7-T1 and the presence or absence of a congenital variant of C7 (Table 1). However, cases were more likely to have severe modelling of the APs compared with control horses, p=0.04, RR: 1.97 (CI: 1.22, 3.18).

The APs of T1-T2 could not be evaluated in five horses. Modelling of the APs at T1-T2 was only identified in 10 horses. There was

no difference between the degree of modelling of the APs at T1–T2 and the presence or absence of a congenital variant of C7. There was no significant difference in the degree of modelling of the APs in control horses versus cases; however, moderate (n=1) or severe (n=1) modelling was only observed in cases.

# 3.4 | Ventral buttressing

There was no significant difference in the presence of ventral buttressing at C6–C7 (Figure 2A–C) in horses with or without ventral laminae present on C7. Ventral buttressing was observed in a smaller proportion of cases (44, 48.7%) compared with control horses (77, 60.6%), p=0.03, RR: 0.71 (CI: 0.53, 0.97). Ventral buttressing at C7–T1 was

Presence of congenital variants						
AP modelling	Total number	Yes (n = 54)	No (n = 169)	RR	95% CI	p Value
C6-C7						
Normal	24 (10.8%)	7 (13.0%)	17 (10.1%)	ref	ref	ref
Mild	67 (30%)	17 (31.5%)	50 (29.6%)	0.87	0.41-1.84	0.7
Moderate	84 (37.7%)	19 (35.2%)	65 (38.5%)	0.78	0.37-1.62	0.5
Severe	48 (21.5%)	11 (20.4%)	37 (21.9%)	0.79	0.35-1.77	0.6
C7-T1						
Normal	110 (49.3%)	21 (38.9%)	89 (52.7%)	ref	ref	ref
Mild	63 (28.3%)	16 (29.6%)	47 (27.8%)	1.33	0.75-2.36	0.3
Moderate	40 (17.9%)	14 (25.9%)	26 (15.4%)	1.83	1.04-3.25	0.05
Severe	10 (4.5%)	3 (5.6%)	7 (4.1%)	1.57	0.57-4.37	0.4
T1-T2						
Normal	206 (94.5%)	50 (96.15%)	156 (93.98%)	ref	ref	ref
Mild	10 (4.6%)	2 (3.9%)	8 (4.82%)	0.82	0.23-2.91	1
Moderate	1 (0.5%)	1 (0.6%)	0 (0%)	-	-	1
Severe	1 (0.5%)	1 (0.6%)	0 (0%)	-	-	1

the presence of one or two ventral laminae (congenital variants) on the seventh cervical vertebra (C7) and the degree of modelling (normal, mild, moderate, severe) of the articular processes (APs) of the sixth cervical (C6) to second thoracic (T2) vertebrae for 223 Warmblood horses, expressed as relative risks (RR) and 95% confidence intervals (CI), compared with normal (reference = ref).

*Note*: There were no significant *p* values.

**TABLE 2** The severity of modelling (normal, mild, moderate and severe) of the articular processes (APs) of the sixth cervical (C6) to second thoracic (T2) vertebrae in 127 control horses and 96 case horses with neck pain and/or stiffness, neck-related forelimb lameness or ataxia and proprioceptive deficits.

AP modelling	Total number	Case (n = 96)	Control (n = 127)	RR	95% CI	p Value
C6-C7						
Normal	24 (10.8%)	8 (8.3%)	16 (12.6%)	ref	ref	ref
Mild	67 (30%)	24 (25.0%)	43 (33.9%)	1.07	0.56-2.06	8.0
Moderate	84 (37.7%)	33 (34.4%)	51 (40.2%)	1.18	0.63-2.2	0.6
Severe	48 (21.5%)	31 (32.3%)	17 (13.4%)	1.94	1.06-3.54	0.01
C7-T1						
Normal	110 (49.3%)	39 (40.6%)	71 (55.9%)	ref	ref	ref
Mild	63 (28.3%)	29 (30.2%)	34 (26.8%)	1.3	0.9-1.88	0.2
Moderate	40 (17.9%)	21 (21.9%)	19 (15.0%)	1.48	1-2.18	0.06
Severe	10 (4.5%)	7 (7.3%)	3 (2.4%)	1.97	1.22-3.18	0.04
T1-T2						
Normal	206 (94.5%)	83 (91.2%)	123 (96.9%)	ref	ref	ref
Mild	10 (4.6%)	6 (6.6%)	4 (3.2%)	1.49	0.87-2.54	0.3
Moderate	1 (0.5%)	1 (1.1%)	0 (0%)	2.48	2.1-2.93	0.4
Severe	1 (0.5%)	1 (1.1%)	0 (0%)	2.48	2.1-2.93	0.4

Note: Results are expressed as relative risks (RR) with 95% confidence intervals (CI), compared with normal (ref = reference). Significant p values are highlighted in bold.

observed in only 24 horses (10.8%). There was no significant difference between the presence or absence of a congenital variant of C7 and the presence of ventral buttressing, or between the prevalence of ventral buttressing in cases and controls. Ventral buttressing at T1-T2 was only observed in 4 of 219 horses, none of which had a congenital variant of C7. There was no significant difference between control horses and cases.

# 3.5 | Intervertebral foramina

The intervertebral foramen at C6-C7 was narrowed in 125 horses, 81 (63.8%) control horses and 44 (45.8%) cases (Figure 2B-D). The intervertebral foramen at C6-C7 was obscured in 41 horses (Figure 2A), but only in 7 (5.5%) control horses compared with 34 (35.4%) cases. The intervertebral foramen was more likely to be

obscured in cases than in control horses, p < 0.0001, RR: 2.63 (CI: 1.75, 3.94). There was no significant difference in the frequency of narrowing or obscuring of the intervertebral foramen between the presence or absence of a congenital variant of C7.

The intervertebral foramen at C7-T1 was narrowed in 64 horses (control horses 34, 26.8%; cases 30, 31.3%) and obscured in 9 (controls 2, 1.6%; cases 7, 7.3%); there was no difference in frequency comparing horses with and without ventral laminae on C7. There was no significant difference between control horses and cases.

The intervertebral foramen at T1–T2 was narrowed in two horses in both cases. Cases were more likely to have narrowing compared with control horses p=0.04, RR: 4.91 (Cl: 3.60, 6.68). However, the presence of a narrowed intervertebral foramen was not significantly different in horses with or without a ventral lamina on C7.

#### 3.6 Other abnormalities associated with the APs

There was an osseous spur on the ventral aspect of the APs of C6–C7 in 12 horses (controls 2, 1.6%; cases 10, 10.4%) (Figure 3A). Cases were more likely to have a spur than control horses, p=0.01, RR: 2.04 (Cl: 1.51, 2.76). However, the prevalence of a spur was not different between horses with or without a congenital variant of C7. Comparison of lateral–lateral and oblique images confirmed that these spurs were usually bilateral, although they could be asymmetrical in size. A smoothly rounded osseous fragment was observed in the C6–

C7 APJs in five horses, two controls (1.6%) and three cases (3.1%) (Figure 2E). There was no significant difference between controls and cases and the presence or absence of a congenital variant of C7. A fracture of one of the APs at C7-T1 was present in one control horse. A smoothly rounded osseous fragment was observed in the APJs at C7-T1 in four horses (2.4%) with no congenital variant of C7 (Figure 2D), and four horses (7.4%) with a congenital variant of C7. The difference was non-significant. Five horses (3.9%) were controls and three (3.1%) were cases.

# 3.7 | Alignment of C6 to T2

The cranial aspect of the vertebral body of C7 was displaced dorsally in one control horse (0.8%) and six cases (6.3%) and ventrally in four controls (3.2%) and in 39 cases (40.6%) (Figures 1C,D and 3B). Cases were more likely to have dorsal (p < 0.001, RR: 2.91 [CI: 1.99, 4.25]) or ventral displacement (p < 0.001, RR: 3.08 [CI: 2.4, 3.95]) compared with controls. There was no significant difference in the frequency of C6–C7 malalignment in horses with and without congenital variants of C7.

The cranial aspect of the vertebral body of T1 was displaced dorsally in 12 controls (9.5%) and in 14 (14.6%) cases; there was no significant difference between controls and cases. The cranial aspect of the vertebral body of T2 was displaced ventrally in one control horse (0.8%) and 11 cases (11.8%). Cases were more likely to have ventral displacement compared with controls, p = 0.0003, RR: 2.45 (CI: 1.9,



FIGURE 3 (A) Lateral–lateral radiographic image of the sixth cervical (C6) to first thoracic (T1) vertebrae of a case horse. Cranial is to the left. There is a transposition of one ventral lamina from C6 to C7, which extends less than half the length of the vertebral body. There are bony spurs on the ventral aspect of the articular process of C5–C6 and C6–C7 (arrows). Comparison with oblique images determined that these were bilateral but were asymmetrical in size. Comparison with osseous specimens of other horses indicates that these are probably ventral periarticular osteophytes on the cranial APs of C6 and C7, respectively. (B) Lateral–lateral radiographic image of the fifth (C5) to seventh (C7) cervical vertebrae of a case with mild ataxia, proprioceptive deficits and weakness. Cranial is to the left. There is dorsal displacement of the head of the vertebral body of C7. The caudal endplate of the vertebral body of C6 is long relative to the dorsoventral height of the head of C6. The intervertebral joint space width of C6–C7 is narrower ventrally than dorsally. There is an asymmetrical enlargement of the articular processes of C6–C7. The head of the vertebral body of C6 is slightly displaced ventrally and the intervertebral joint space width of C5–C6 is narrower ventrally than dorsally. This mass confirmed in a more caudal image centred on the joint. There are separate centres of ossification of the caudoventral aspect of the vertebral body of C6 (arrow). (C) Lateral-lateral radiographic image of the sixth cervical (C6) to first thoracic (T1) vertebrae of a case with neck-related forelimb lameness. Cranial is to the left. Both ventral laminae of C6 are transposed to the vertebral body of the seventh cervical (C7) vertebra. There are two mineralised opacities dorsal to the C6–C7 intervertebral articulation (arrow) and subtle modelling of the ventrocaudal aspect of the vertebral fossa of C6. There is a mild asymmetrical enlargement of the articular processes at C6–C7 and C7–T1.

3.16). The prevalence of dorsal or ventral displacement was not significantly different in horses with or without a congenital variant of C7. The cranial aspect of the vertebral body of T2 was displaced dorsally in 3 of 126 (2.4%) controls and 2 of 78 (2.6%) cases.

Spondylolisthesis (subluxation) at both C6–C7 and C7–T1 was more likely to occur in cases (18, 18.5%) than in controls (0), p < 0.0001, RR: 2.63 (CI: 2.21, 3.13). Cases were more likely to have spondylolisthesis and modelling of APs (11, 11.5%) compared with controls (0), p < 0.0001, RR: 2.49 (CI: 2.12, 2.94).

#### 3.8 | Intervertebral articulations

Discospondylosis at C6-C7 was seen in six cases, none of which had a congenital variant of C7. Cases were more likely to have discospondylosis than controls, p = 0.01, RR: 2.41 (CI: 2.06, 2.82). Discospondylosis at C7-T1 was seen in 10 horses, 7 (4.1%) of which had no congenital variant compared with 3 (5.6%) with a ventral lamina on C7. This difference was not significant. Discospondylosis predominated in cases (9, 9.4%) compared with controls (1, 0.8%). Cases were more likely to have discospondylosis than controls, p = 0.003, RR: 2.2 (CI: 1.70, 2.86). At T1-T2, discospondylosis was observed in 10 horses, eight of which had no congenital variant of C7. Cases were more likely to have discospondylosis (10/64, 15.6%) compared with controls (0), p < 0.001, RR: 3.3 (CI: 2.67, 4.17). In addition, two cases (forelimb lameness and neck stiffness, respectively) (Figure 3C) and one control horse had small rounded mineralised opacities dorsal to an intervertebral symphysis (C6-C7 ± C5-C6), which had no other detectable radiological abnormality. Two of the three horses had a congenital variant of C7.

#### 3.9 | Clinical diagnoses and radiological findings

The presence or absence of a congenital variant of C7 for cases and control horses are summarised in Table 3. There was no significant difference between the type of case (neurological, neck-related fore-limb lameness, neck pain and/or stiffness) and the presence or absence of a congenital variant of C7, although cases were less likely to have congenital variants than control horses, p = 0.02, RR: 0.63 (CI: 0.4, 0.97).

#### 4 | DISCUSSION

Contrary to the first hypothesis there was a higher prevalence of congenital variants of C6 and C7 in control horses compared with cases. Contrary to the second hypothesis, there was no difference in the presence or absence of modelling of the APs of C6–C7 or C7–T1 between horses with congenital variants of C6 and C7 compared with horses without congenital variants of C6 and C7. Contrary to the third hypothesis, the prevalence of modelling of the APs of C6–C7 and C7–T1 was similar in cases compared with control horses. However, in accordance with the fourth hypothesis cases were more likely to have severe modelling of the APs compared with control horses.

Congenital variants of C6 and C7 have previously been referred to as equine complex vertebral malformation, <sup>19</sup> likening the 'condition' to the lethal autosomal recessive disease, complex vertebral malformation syndrome in Holstein calves. <sup>29</sup> The conditions have no similarity, with no alterations of the ventral laminae of C6 or C7 recorded in affected calves. Moreover, in the current study, there was no relationship between congenital variants of C6 and C7 and other radiological abnormalities of the cervicothoracic region.

The prevalence of congenital variants of C7 in the current study (24.2% of all horses, 29.2% of control horses, 16.7% of cases) was similar to previous radiological studies in both mixed breed populations (24/100, 24%<sup>6</sup>; 24/116, 20.7%<sup>17</sup>; 26/271, 13.3%<sup>15</sup>; 3/25, 12.0%<sup>8</sup>; 26/78, 33.3%<sup>16</sup>) and Warmbloods (overall 108/377, 28.6%; cases 58/245, 27.3%; 50/132, 38.0% control horses)<sup>7</sup>; 10/28, 35.7% yearling Dutch Warmbloods<sup>30</sup>; 23/104, 22.1% mature showjumpers<sup>22</sup> and 168/664, 25.3% mature Warmbloods. 31 In accordance with the majority of previous clinical studies in either Warmbloods<sup>7,31</sup> or mixed breed populations 16 there was no relationship between the presence of congenital variants of the ventral laminae of C7 and the presence of neck-related clinical signs compared with control horses. However, an association between neck pain and congenital variants of the ventral laminae of C7 was observed in a mixed breed population, although no association between neck-related forelimb lameness or neurological abnormalities and congenital variants of the ventral laminae of C7 was identified.<sup>6</sup> In addition, in a second mixed breed study there was an association between congenital variants of the ventral laminae of C7 and neck pain or neurological signs. 17

In contrast to previous studies, the current study employed a predefined clinical protocol for the prospective evaluation of all horses. In

**TABLE 3** Summary of the frequency of the presence of one or more ventral laminae on the seventh cervical vertebra (C7) (= congenital variant) in control horses (n = 127) and cases (n = 96) with neck pain and/or stiffness, neck-related forelimb lameness or ataxia and proprioceptive deficits.

	No congenital variant, $n = 169$	Congenital variant of C7, $n = 54$	RR	95% CI	p Value
Control horses	89, 52.7%	38, 70.4%	ref		
Neck pain/stiffness	14, 8.3%	4, 7.4%	0.74	0.30-1.83	0.5
Forelimb lameness	27, 16.0%	4, 7.4%	0.43	0.17-1.12	0.05
Neurological dysfunction	39, 23.1%	8, 14.8%	0.57	0.29-1.13	0.09

Note: Results are expressed as relative risks (RR) with 95% confidence intervals (CI), compared with control horses (ref = reference). There were no significant p values.

addition, the presence of congenital variants of the ventral laminae of C7 was compared with a larger number of radiological abnormalities from C6 to T2 compared with most previous studies. There was no association between the presence of congenital variants of the ventral laminae of C7 and any of these radiological abnormalities. It, therefore, appears that despite the observation that anatomical variants of the ventral laminae of C6 and C7 may be associated with variations in longus colli muscle insertions on the ventral aspect of the caudal cervical and cranial thoracic vertebrae and thus altered and potentially asymmetrical biomechanical forces, 14 this does not necessarily translate to clinically significant disease. Longus colli principally acts both as a flexor and an intersegmental stabiliser. The flexor action of longus colli is opposed by the principal extensor muscles, splenius, spinalis cervicis. semispinalis capitis and longissimus capitis and cervicis. 32-34 The segmental stabilisation provided by longus colli is also supported by the more dorsally located multifidus cervicis<sup>34</sup> and laterally the intertransversarius muscles (dorsalis cervicis, medii cervicis and ventralis cervicis).<sup>27,35</sup> Moreover, the longus colli muscle has ventral, deep and medial lavers with multiple insertion sites on to each vertebra from C2 to C6; a large muscle belly extends caudally as longus thoracis to T5 or T6, with intermediate attachments to the ventral longitudinal ligament overlying intervertebral disks, and into the joint capsules of the medial costovertebral articulations from T1 to T4-T5.36 Although it has been suggested that altered function of longus colli may result in instability, and thus asymmetry of APs and OA, 14 there have been no longitudinal studies to support this hypothesis. In the current study, asymmetry of the APs at C6-C7 was seen with similar frequency in horses with or without a congenital variant of C7 but was observed more frequently in cases compared with control horses.

Moreover, extension of the caudal cervical–cranial thoracic region has a greater effect on the relative positions of adjacent vertebrae and the dimensions of the intervertebral foramina compared with flexion<sup>37</sup> and may influence modelling of the APs.<sup>35</sup> Modelling ± asymmetry of caudal cervical APs is a common finding at post-mortem examinations of horses with and without neck-related clinical signs.<sup>11,35</sup> It has been suggested that the abrupt transition from a freely moveable cervical region to a relatively fixed cranial thoracic region,<sup>38</sup> combined with a reduction in the articular surface area of the APs from C7–T2,<sup>35</sup> may predispose to stress concentration, especially during extension, resulting in modelling changes of the APs.<sup>38</sup>

In the current study, cases were more likely to have severe modelling of the APs of C6–C7 or C7–T1 compared with control horses, as observed in a mixed breed study. Moderate (n=1) or severe (n=1) modelling of the APs of T1–T2 were only observed in cases. In a post-mortem study, there were different morphological changes in the cranial and caudal APs. Osteophytes, periarticular lipping and joint capsule entheseophytes predominated on cranial APs, whereas caudal APs were flattened and modelled to accommodate bony proliferation on opposing articular surfaces during extension.  $^{35}$ 

In a previous age-matched case-control study of 30 cases and 30 control horses, more severe abnormalities of the APs of C5-C6 and C6-C7 were detected in cases than controls, as in the current study, however the criteria for designation of cases were not clearly

defined, and there was no standardised method of clinical assessment of cases and control horses. 10 In a study which defined the region of the neck in which problems were thought to be present (cranial, mid or caudal) rather than the type of clinical problem, higher radiological grades were assigned to the APJs of C5-C6, C6-C7 and C7-T1 in the group with complaints in the caudal half of the cervical region compared with control horses or those with a cranial or mid neck problem. 31 However, case definitions were thoroughly described and the study could not have been replicated. In contrast, in a study of competing showjumping horses, 67.3% had abnormalities of the APJs of C6-C7, with 44 (42.3%) graded as mild and 26 (25.0%) graded as moderate or severe.<sup>22</sup> The presence of focal pain on pressure of the neck or reduced range of motion were not correlated with the radiological grade. Moreover, there was a significant positive correlation between the highest level at which a horse had competed and the severity of the radiological grade of OA of the APJs of C6-C7.

A study using computed tomography (CT) reported that modelling (enlargement) of APs could be present without other radiological signs of OA,<sup>25</sup> such as alteration of joint space width and articular margins, and hypoattenuation or hyperattenuation of subchondral bone. It was implied that modelling alone was not necessarily consistent with OA. However, modelling could result in a compromise of the intervertebral foramina.<sup>26</sup> Moreover, severe modelling with abaxial lipping might mechanically inhibit lateral bending and axial rotation.<sup>35</sup>

In the current study, the prevalence of ventral buttressing at C6–C7, C7–T1 or T1–T2 was not different in cases versus control horses or in those horses with or without congenital variants of C7. Ventral buttressing has previously been described as a potentially incidental finding. The presence of osseous fragments associated with the APJs was similar in frequency in control horses and cases in the current study. In a CT study, there was a significant difference in the prevalence of osseous fragments in ataxic horses compared with control horses, but no difference between control horses and those with forelimb lameness or neck pain and/or stiffness. In three other CT studies, one or more osseous fragments associated with APJs were identified in <10% of 180 horses, 39 and 54 (24%) and 11 of 51 (22%) horses, but the majority were present together with other osseous abnormalities and their clinical significance was questioned. Surgical removal of mineralised pieces has been described in a small number of horses with variable success. 12,43

Osseous spurs on the ventral aspect of the APs of C6–C7 occurred more frequently in cases compared with control horses. The prevalence of such spurs has not previously been described to our knowledge. Comparison with osseous specimens indicates that these are probably cranioventral periarticular osteophytes on the cranial APs, as also previously described in a post-mortem study.<sup>35</sup> Collectively, these studies indicate that the presence of modelling of the APs alone is not synonymous with the likelihood of current clinical signs, <sup>10.11,17,22,25,26,35,38</sup> but severe modelling, especially when combined with other osseous abnormalities, is more likely to be of clinical significance than mild or moderate modelling. The current study also shows the potential importance of examination of the cranial thoracic vertebrae in a horse with clinical signs referrable to the cervicothoracic junction.

The intervertebral foramina are not easy to assess in twodimensional lateral-lateral radiographic images because they have an oblique orientation, and the left and right sides are superimposed. Anatomical studies indicate that narrowing of the caudal aspect of the foramina is more likely to create nerve root compression than cranial narrowing, especially when the caudal cervical region is in extension.<sup>37</sup> Additional information may be available from oblique radiographic images, but standardisation of image acquisition is challenging despite the availability of excellent guidelines. 44 Moreover, in CT studies, the side of maximum narrowing does not always coincide with the laterality of clinical signs, making image interpretation potentially challenging.<sup>26</sup> In the current study, the intervertebral foramina were more likely to be obscured at C6-C7 in cases compared with control horses; alteration in size of the intervertebral foramen at C7-T1 did not differ between cases and controls. Narrowing of the intervertebral foramen at T1-T2 was only seen in cases. The authors suggest that the acquisition of an additional lateral-lateral radiographic image with the caudal neck region in extension may give additional information about the potential for obscurement of the intervertebral foramina.

Mild dorsal displacement of the cranial aspect of T1 was seen with similar frequency in cases and control horses, whereas ventral displacement was more likely in cases. If malalignment is mild, the authors advise that repeat radiographs should be acquired with an alteration of the neck position to determine that the change is genuine and repeatable. However, at C6–C7, dorsal or ventral spondylolisthesis of the cranial aspect of C7 was seen more frequently in cases than controls, as previously documented, <sup>17</sup> and at T1–T2, ventral displacement of the cranial aspect of T2 occurred more often in cases than controls. Dorsal or ventral spondylolisthesis was often seen in association with mild alterations in intervertebral disc space width.

Intervertebral disc disease and discospondylosis at C6-C7, C7-T1 and T1-T2 were seen considerably more frequently in cases compared with control horses. The caudal cervical and cranial thoracic region is a predilection site for lesions detected using radiography. The grading of different types of lesions was outside the remit of this study, but the radiological abnormalities varied greatly among horses, as previously observed.9 A technique for objective measurement of disc space width compared with the craniocaudal length of the cranial vertebral body has been described<sup>30</sup> but was not used in the current study. The limitations of conventional radiography versus high-field MRI for the detection of intervertebral disc pathology without associated osseous abnormalities have been well-documented.<sup>45</sup> In the current study small rounded mineralised opacities were identified dorsal to an intervertebral articulation without other detectable radiological abnormalities of the joint in three horses. Such opacities are considered likely to reflect intervertebral disc pathology. Similar hypoattenuating opacities have been identified in the dorsal aspect of a dorsally protruding intervertebral disc on CT images at C6-C7.<sup>39</sup>

# **5** | LIMITATIONS

The control horses and cases were not precisely age-matched, the cases being, on average, 1 year older than the control horses. This is

of unlikely biological relevance to the results. Previous studies have shown variability in radiological assessment among observers. Agreement between four clinicians' grades of OA of the APJs for C5-C6 and C6-C7 was moderate for lateral-lateral radiographs (0.49) of 17 horses, 46 similar to the moderate agreement observed by Koenig for C6-C7 (kappa range: 0.5-0.6). 10 The agreement among observers for cone-beam CT was lower (0.36). For both lateral-lateral radiographs and cone-beam CT, agreement was better for Grade 3 lesions compared with Grade 1 lesions. However, when lateral-lateral radiographs were compared with cone-beam CT, agreement was fair (kappa 0.38, 95% CI: 0.23, 0.53).46 In another study which compared the interpretation by three radiologists, agreement ranged from 0.37 to 0.61.<sup>22</sup> Before the commencement of the current study the radiological grading system was agreed and applied with consensus by two assessors. Dividing the evaluation into many well-defined categories potentially enhanced repeatability and accuracy of interpretation, but objective assessment of this fell outside the remit of the study. The radiological grading system proposed by Criins and Broeckx<sup>31</sup> was not used because we wished to compare the presence or absence of congenital variants of C7 with a large variety of distinct radiological abnormalities. Oblique views targeted to evaluate the morphology of congenital variants of the ventral laminae of C7<sup>47</sup> were not acquired: the overseeing ethical review committees ruled that only images required as part of a routine clinical assessment could be obtained. Radiological assessment of the first ribs was not performed routinely, although it is acknowledged that congenital variants have occasionally been associated with clinical signs. 48-50 sometimes in association with congenital variants of C7. If both first ribs had been absent or congenital fusion of the first and second ribs had been present, it would have been recorded, but vestigial first ribs, the absence of one first rib or morphological variants of the distal two-thirds of the ribs may have been missed. In a post-mortem CT study, the absence of first ribs (n = 1) or the presence of vestigial first ribs (n = 2) were identified in three mature Warmblood horses (3/78 [3.8%] horses examined), each of which had congenital variants of C7.16

The current study was not a longitudinal study, and the future development of neck-related clinical signs in control horses cannot be predicted. Moreover, the horses were not necessarily representative of the Warmblood population as a whole in either the United Kingdom or the USA, or in continental Europe, where Warmblood breeding predominates. The use of a referral population of horses created inherent bias but did permit the standardisation of clinical assessments. Some of the control horses were not clinically normal, but lameness or other performance problems were abolished by the use of diagnostic anaesthesia with no residual clinical signs referrable to the cervicothoracic region.

#### 6 | CONCLUSIONS

There was no association between the presence of congenital variants of C7 and any of the radiological observations. Congenital variants occurred less frequently in cases compared with control

horses, and there was no association between the presence or absence of a congenital variant and the type of case (neurologic, neck pain or stiffness, or neck-related forelimb lameness). The presence of severe modelling of APs, obscuring of intervertebral foramina, spondylolisthesis or discospondylosis was more likely to be observed in cases compared with control horses. Radiographic assessment of horses with a suspected cervicothoracic region lesion should include the cranial thoracic vertebrae to at least T2.

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#### CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicting interests.

#### **AUTHOR CONTRIBUTIONS**

Sue Dyson: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; data curation; supervision; formal analysis; project administration. Kathryn Phillips: Validation; writing – review and editing; software; formal analysis; data curation. Shichen Zheng: Formal analysis; data curation; writing – review and editing; software; validation. Monica Aleman: Conceptualization; investigation; funding acquisition; methodology; validation; visualization; writing – review and editing; formal analysis; data curation; supervision; writing – original draft; project administration.

#### **DATA INTEGRITY STATEMENT**

Sue Dyson and Monica Aleman had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

#### ETHICAL ANIMAL RESEARCH

The study was approved by the Clinical Ethical Review Committee of the Animal Health Trust (AHT 33-2016) and the University of California Davis Institutional Animal Care and Use Committee #19891.

#### **INFORMED CONSENT**

Horse owners gave informed consent.

#### **PEER REVIEW**

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/evj.14127.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request: Open sharing exemption granted by editor for this clinical report.

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#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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