EX VIVO COMPUTED TOMOGRAPHIC EVALUATION OF MORPHOLOGY VARIATIONS IN EQUINE CERVICAL VERTEBRAE

STEFANIE VERAA, WILHELMINA BERGMANN, ANTOON-JAN VAN DEN BELT, INGE WIJNBERG, WILLEM BACK

Diagnostic imaging is one of the pillars in the clinical workup of horses with clinical signs of cervical spinal disease. An improved awareness of morphologic variations in equine cervical vertebrae would be helpful for interpreting findings. The aim of this anatomic study was to describe CT variations in left–right symmetry and morphology of the cervical and cervicothoracic vertebrae in a sample of horses. Postmortem CT examinations of the cervical spine for horses without congenital growth disorders were prospectively and retrospectively recruited. A total of 78 horses (27 foals, 51 mature horses) were evaluated. Twenty-six horses (33.3%) had homologous changes in which a transposition of the caudal part of the transverse process (caudal ventral tubercle) of C6 toward the ventral aspect of the transverse process of C7 was present ($n = 10$ bilateral, $n = 12$ unilateral left-sided, $n = 4$ unilateral right-sided). There was one horse with occipito-atlantal malformation, two horses with rudimentary first ribs bilaterally, and one horse with bilateral transverse processes at Th1, representing homeotic (transitional) vertebral changes. Chi-square tests identified no significant differences in the number of conformational variations between the group of mature horses with or without clinical signs ($P = 0.81$) or between the group of mature horses and the group of foals ($P = 0.72$). Findings indicated that, in this sample of horses, the most frequently identified variations were homologous variations (transposition of the caudal part of the transverse process of C6–C7) in the caudal equine cervical vertebral column. Homeotic (transitional) variations at the cervicothoracic vertebral column were less common.

Key words: cervical vertebral column, homeotic, homologous, horse, transitional vertebra.

Introduction

DIAGNOSTIC IMAGING IS AN ESSENTIAL tool in the clinical work up of horses with signs that may be related to the cervical spine such as spinal ataxia, decreased mobility, and lameness.[1–4] It is therefore important to appreciate the possibility of anatomic vertebral variations when interpreting diagnostic images of horses. The deviation from traditional vertebral patterning can be a change in numbers in one section with preservation of total vertebral count (*homeotic variation)* such as seen in transitional vertebrae, but may also consist of a change in the total number of vertebrae (*meristic variations*). Changes in merely one subunit with preservation of the traditional number and identity of the vertebrae can occur when only size and/or shape of vertebrae are altered (*homologous variations*),[5] an example is the transposition of the caudal part of C6–C7 in horses.[6]

Anatomical variations of the equine cervical vertebral column have been reported in the early 1900s.[7,8] The prevalence of cervical anatomical variations in larger populations has recently been described in the literature.[6,9,10] There is one retrospective study based on radiographic examinations in which anatomical variations of the spinous and transverse processes in the caudal cervical vertebrae and the first thoracic vertebrae were found in, respectively, 24.9% and 13.3% of the horses.[10] An incidence of 38% of congenital malformations of the sixth and seventh vertebrae was recorded during dissection in a thoroughbred-based population of horses in another study[6] and associated variations in longus colli muscle attachment at this level.[11] A prevalence of 24% anomalous C6 was recorded in a recent study with a predilection for Warmblood breeds as these accounted for almost 80% of the affected and included horses.[12] Advanced imaging modalities such as computed tomography (CT)[13–15] and magnetic resonance imaging (MRI)[13–17] are increasingly being applied in the evaluation of horses with suspected cervical spinal disease. However, little published information is currently available

From the Division of Diagnostic Imaging, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 110, 3584 CM (Veraa, van den Belt), Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL (Bergmann), and Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 114, 3584 CM (Wijnberg, Back), Utrecht, The Netherlands, and Department of Surgery and Anaesthesiology of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820, Merelbeke, Belgium (Back).

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Address correspondence and reprint requests to Stefanie Veraa, at the above address. E-mail: S.Veraa@uu.nl

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on CT and MRI characteristics of equine cervical vertebral morphologic variations.

The purpose of the present study was to assess the presence and the morphological appearance of anatomical variations of the cervical vertebral column and cervicothoracic junction using ex vivo CT in a group of horses. We hypothesized that, given the reported prevalence of 13.3% using radiography and 38% in dissected thoroughbreds, the prevalence of these vertebral variations in the equine vertebral column as diagnosed by CT would be between 13% and 38% with the anatomy generally being as described in the classic anatomical references.[18,19] We also hypothesized that the prevalence of anatomical variations would be equal in the groups of horses with and without clinical signs related to the cervical column.

Material and Methods

Horses

The study was an anatomic design. Equine cadaver necks were prospectively collected for CT examination after owner consent or retrospectively retrieved from horse cadavers included in a different study.[20] All available ex vivo CT examinations of the cervical vertebral column during the period of January 2009 to July 2014 in the Picture Archiving and Communication System (PACS) of the Division of Diagnostic Imaging, Faculty of Veterinary Medicine of Utrecht University were considered for inclusion. Horses were excluded if they had a known congenital growth disorder or if there was incomplete evaluation of the cervical vertebral column in the scan. Medical records for included horses were retrieved and available clinical data (breed, age, clinical signs) were recorded.

Specimen Collection and CT Scanning Techniques

The cervical vertebral column was disarticulated at the level of thoracic vertebrae (Th) 2–3, the head disarticulated (when possible) at the occipito-atlantal junction and a large part of the soft tissues surrounding the vertebral column was removed in horses before CT examination at the Department of Pathobiology of the Faculty of Veterinary Medicine, Utrecht University by a board-certified pathologist (W.B.). Dissection was not performed in eight foals. In one horse, dissection was not possible at the occipito-atlantal junction and the head remained attached. Computed tomography scans were made from the first cervical vertebra (C1) to the second thoracic vertebra (Th2). All CT examinations were performed with a single slice CT scanner (Philips Secura, Eindhoven, The Netherlands) using the following scanning protocol: scanning parameters 100 kVp, 220 mA, 0.7 s tube rotation time and 2- to 3-mm thick contiguous slices. A bone algorithm was applied. A standard matrix of 512 × 512 pixels was used. The field of view (FOV) varied between the examinations depending on the size of the cadaver neck.

Data Analysis

The CT images of the cervical vertebrae were evaluated by a board-certified veterinary radiologist (S.V.), who was not aware of the in vivo clinical status of the horse at that time. Images were retrieved from the available PACS system (Impax, version 6.5.2.657, Agfa Healthcare N.V., Mortsel, Belgium). Multiplanar reconstruction (MPR) and 3D volume rendering of the vertebrae were performed. Left–right symmetry and anatomy (such as vertebral body, lamina, pedicles, transverse processes, spinous process, and presence of intervertebral disc space) of the cervical vertebrae were evaluated. The vertebral shape and conformation were compared to the images and descriptions of the standard anatomical reference works.[18,19] Variations were considered homeotic if they were positioned only at a section junction (head-cervical and cervical-thoracic) and the vertebra had the characteristics of a transitional vertebra. Variations were considered homologous if the changes were not positioned at a section junction and merely changed the shape of the vertebra without true characteristics of a transitional vertebra.

The horses were assigned to two groups according to the recorded clinical status: without clinical signs or with clinical signs related to the cervical vertebral column. Clinical signs related to the cervical vertebral column recorded were, among others, pain, reduced motion, or spinal ataxia. Clinical examinations were performed by a board-certified veterinary surgeon (W.B.) or board-certified veterinary internist (I.W.). Prevalences of anatomical variations in the groups of mature horses with vs. without clinical signs and the groups of mature horses vs. foals were compared using a chi-square test (IBM SPSS Statistics version 22, IBM, New York). Statistical analyses were completed by the first author (S.V.) and significance was determined using $P < 0.05$.

Results

Horses

Included in this study were 78 horses (65 Dutch Warmblood horses, two Friesians, two Zangersheide Studbook horses, two cross-breed horses, two Westfaler, one Arabian, one Oldenburger, one Trotter, one New Forest Pony, one Appaloosa) including 29 mares, 17 stallions, and 32 geldings; 51 mature horses (median age 6.9 years, range 2.2–22 years); and 27 foals (median age 7 days, range 0–150 days). Thirty mature horses had clinical signs related to cervical vertebral column pathology (such as spinal ataxia or pain) and 21 died of or were euthanized for reasons unrelated to cervical vertebral column pathology (such as lameness due to tendinitis or joint pathology of the limbs). The 27 foals died of or were euthanized for reasons unrelated to cervical vertebral column pathology (such as respiratory distress, viral or bacterial septicemia, and gastric rupture).

The cervical vertebral column of all equine cadavers was examined within 24 h after death. The cervical vertebral columns of 51 of the 78 horses in this study were considered symmetric and conformed to the description in the anatomical references (Figs. 1A, 2A, and 3A). In 27 horses, asymmetry and/or variations of the standard anatomy were detected (Table 1).

Homeotic or Transitional Variations

Homeotic or transitional vertebral variations were seen in four horses. Asymmetric occipito-atlantal fusion, without changes in shape and/or position of the axis, was seen in one (Dutch Warmblood) horse. The presence of bilateral rudimentary ribs at the level of Th1 was noted in two (Dutch Warmblood) horses with concurrent unilateral right-sided and bilateral transverse process changes in C6 and C7, respectively (Fig. 4A and B). In one (Dutch Warmblood) horse, ribs were absent at Th1 and replaced by transverse processes with a shallow split-like indentation in the lateral aspect (Fig. 4C). This horse also had bilateral changes at C6 and C7. No fusion of vertebrae with concurrent loss of intervertebral disc space was seen in any of the horses.

Homologous Variations

In 26 horses (21 Dutch Warmbloods, one Oldenburger, one Westfaler, one Trotter, one Friesian, and one cross-breed) homologous variations were seen. The caudoventral part of the transverse process (caudal-ventral tubercle) was partially ($n = 2$) or completely ($n = 24$) absent unilaterally ($n = 12$, left-sided; $n = 4$, right-sided) or bilaterally ($n = 10$; Figs. 1B and 2B and C). Absence of the ventral part

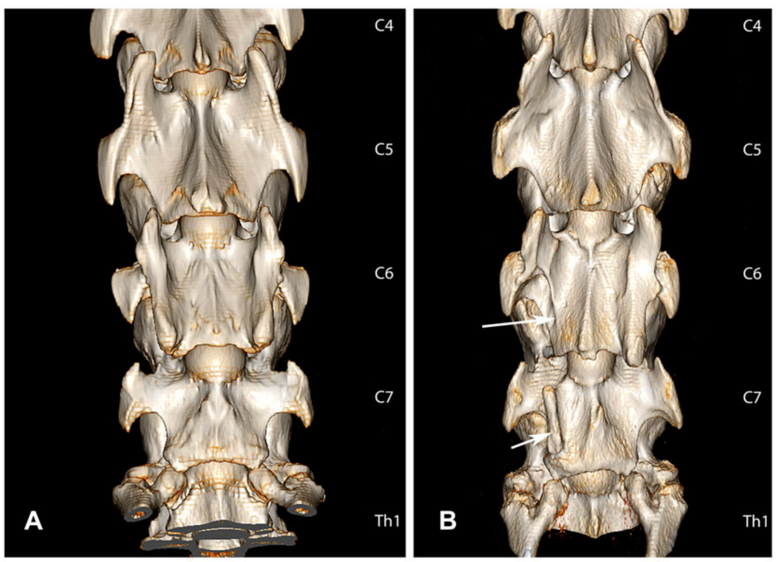

FIG. 1. 3D computed tomography (CT) reconstructions of the caudal cervical vertebral column, ventral aspect. (A) Normal vertebral anatomy, (B) unilateral right-sided homologous vertebral changes at the level of C6 and C7. The ventral part of the transverse process of C6 is not present (long arrow), however, a ventral protuberance is present at the ventral aspect of the transverse process of C7 (short arrow).

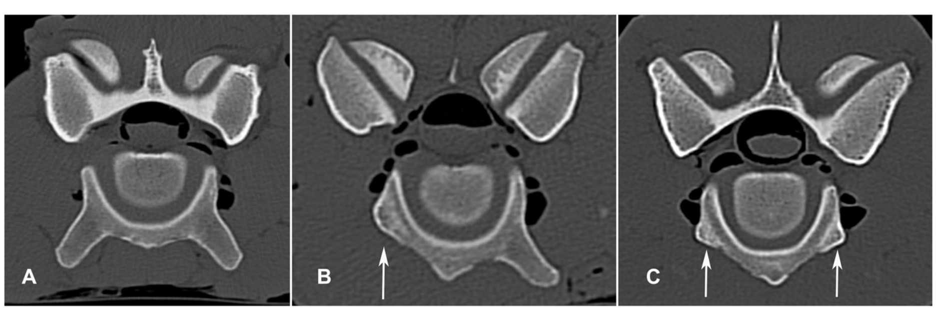

FIG. 2. Transverse CT images at the caudal aspect of C6; right side is at the left side of the image, dorsal is at the top of the image. (A) Normal vertebral anatomy (arrow); (B) unilateral right-sided absence of the ventral part of the transverse process; (C) bilateral absence of the ventral part of the transverse process (arrows).

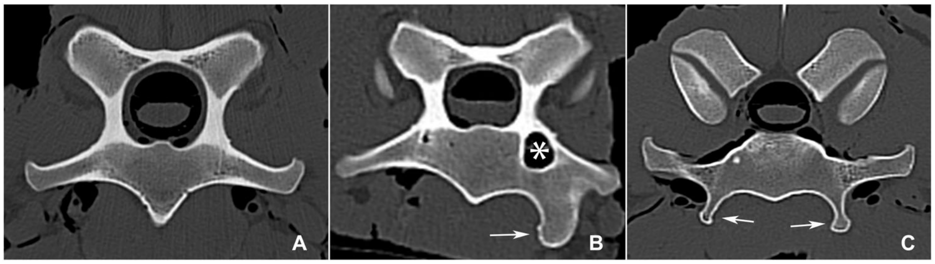

FIG. 3. Transverse CT images at the level of C7; right side is at the left side of the image, dorsal is at the top of the image. (A) Normal vertebral anatomy; (B) unilateral left-sided presence of a ventral protuberance at the transverse process of C7 (arrow) in concurrence with a vertebral foramen at the base of the transverse process (asterisk); (C) bilateral presence of a ventral protuberance at the transverse process of C7 (arrows). This image is slightly more caudally positioned than the images (A) and (B) to allow optimal visualization of the protuberances.

TABLE 1. Numbers of Foals and Mature Horses with Normal and Abnormal Cervical

	Foals Normal Anatomy	Aberrant Anatomy	Mature horses Normal Anatomy	Aberrant Anatomy
Total	19	8	32	19
Breeds	14 DW*, 2 ZS†, 1 Westfaler, 1 Arabian, 1 cross-breed	8 DW*	29 DW*, 1 Friesian, 1 New Forest Pony, 1 Appaloosa	14 DW*, 1 Oldenburger, 1 Westfalen, 1 Trotter, 1 Friesian, 1 cross-breed
Occipito-atlantal fusion		0		1
Bilateral changes C6–7		2		8
Unilateral left-sided changes C6–C7		6		6
Unilateral right-sided changes C6–C7		0		4
Bilateral vertebral foramen C7		1		2
Unilateral left-sided vertebral foramen C7		4		4
Unilateral right-sided vertebral foramen C7		0		3
Rudimentary ribs bilaterally Th1		0		2
Transverse processes bilaterally Th1		0		1
Absent fovea costalis		0		7

*DW, Dutch Warmblood.
†ZS, Zangersheide Studbook.

of the transverse process of C6 was associated in all horses with a protuberance at the ventral aspect of the transverse process of C7. Therefore, a unilateral ($n = 12$, left-sided; $n = 4$, right-sided) or bilateral ($n = 10$) protuberance was present (Figs. 1B and 3B and C). A vertebral foramen was present in the base of four left-sided and six bilateral C7 transverse process ventral protuberances (Fig. 3B).

The absence of a proper fovea costalis in the caudoventral endplate of C7 (Fig. 5) was detected bilaterally in seven horses, which all had bilateral transverse process changes in C6 and C7. Presence of a spinous process at C7 was seen in all adult horses and in 24 foals. In three foals (0 days of age) no spinous process could be seen yet. The spinous processes were ranging in height from 23.6 to 54.8 mm with a median height of 40 mm.

The conformational variations in the caudal cervical vertebral column were seen in 18 of 51 mature horses (35.3%), 11 of 30 (36.7%) mature horses with clinical signs related to the cervical vertebral column and in seven of 21 mature horses (33.3%) without clinical signs related to the cervical vertebral column. The changes were bilateral in six, left-sided in three and right-sided in two of the 11 mature horses that were presented with clinical signs related to the cervical vertebral column. No significant difference in number of conformational variations was noted between the group of mature horses with or without clinical signs (chi-square test, $P = 0.81$).

Eight of 27 foals (29.6%) had conformational variations. No significant difference in number of conformational variations was noted between the group of mature horses and the group of foals (chi-square test, $P = 0.72$).

Discussion

In the present study, homeotic and/or homologous variations of the cervical vertebral column were found in 34.6% (27 of 78) horses, of which 5% (four of 78) had homeotic and 33.3% (26 of 78) had homologous changes. Prevalence of homologous variations was close to 38% and within groups not statistically different, which confirms our hypotheses.

Homeotic vertebral patterning variations (transitional vertebra) were described before in other animals as the presence of occipito-atlantal fusion/occipitalization of the atlas, rudimentary (small or undeveloped) ribs at the first

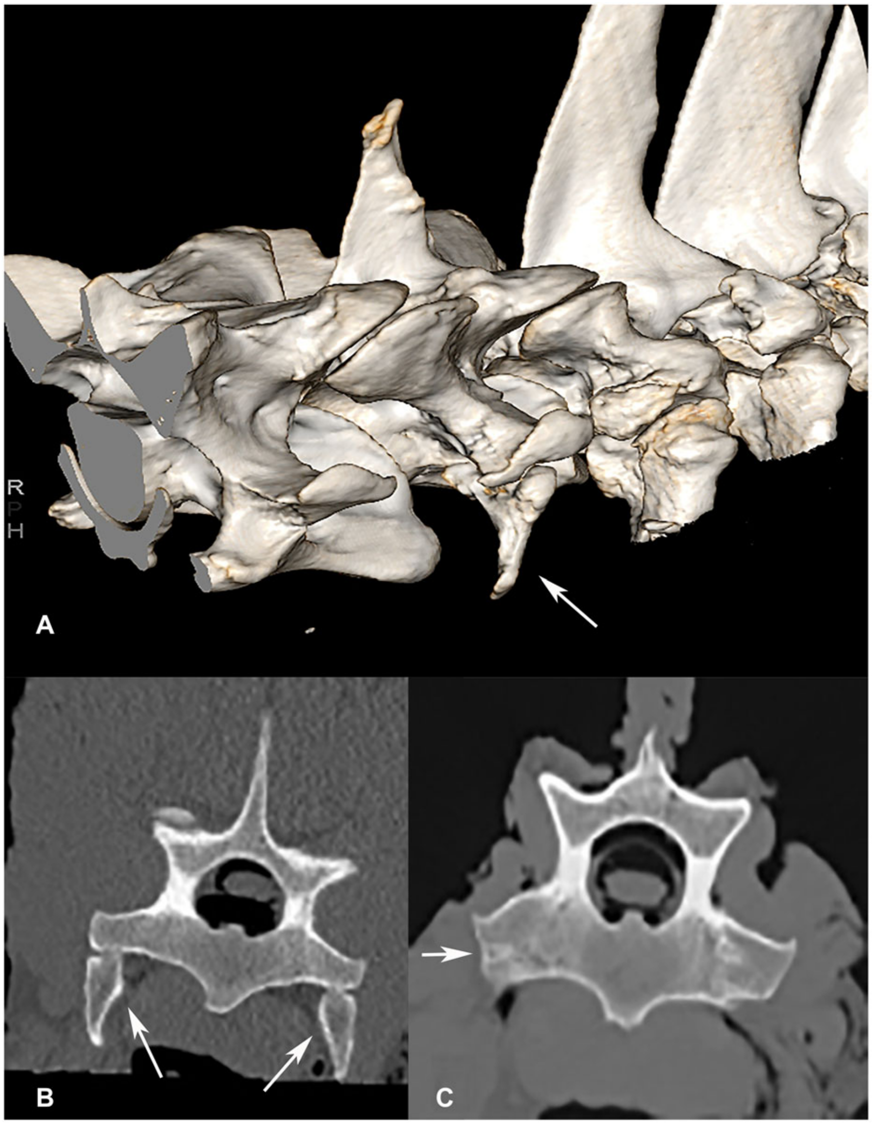

FIG. 4. (A) 3D CT reconstruction of the caudal cervical vertebral column, left craniolateral view. Note the presence of a very short and small first (rudimentary) rib at the ventral aspect of the first thoracic vertebra. (B) Transverse CT image at the level of Th1. Right side is at the left side of the image, dorsal is at the top of the image. Rudimentary ribs are present bilaterally. (C) Transversely reconstructed CT image at the level of Th1. Right side is at the left side of the image; dorsal is at the top of the image. No ribs are present; bilateral transverse processes with a small slit-like opening are visible.

thoracic vertebra, or cervical ribs (small ribs at C7)[5] and detected in four horses of this study.

In one horse occipito-atlantal fusion was found without malformation of the axis or clinical signs. Clinical consequences of occipito-atlanto-axial malformation (OAAM) have been identified in horses and humans depending on the severity of vertebral changes and involvement of the axis.[21–23] There were two mature horses with rudimentary ribs at Th1 and one mature horse in which there were no ribs but transverse processes at Th1, features of homeotic variation and therefore transitional vertebrae. Only one case report on the existence of a rudimentary first rib in a horse was found in literature.[7] In some sloths and human fetuses, the existence of rudimentary or no ribs at the first thoracic vertebra ("posterior" patterning) has been described without known clinical relevance.[5,24] The lack of literature regarding this variation in equine patients might be explained by the inability of analog radiography to image the area of the first thoracic vertebra.

Presence of cervical ribs in humans has been correlated to a higher occurrence of malformations and neoplasia in childhood[24–26] and has been identified in humans with thoracic outlet syndrome (brachial plexus, subclavian artery, and vein compression),[27] a syndrome that has not been

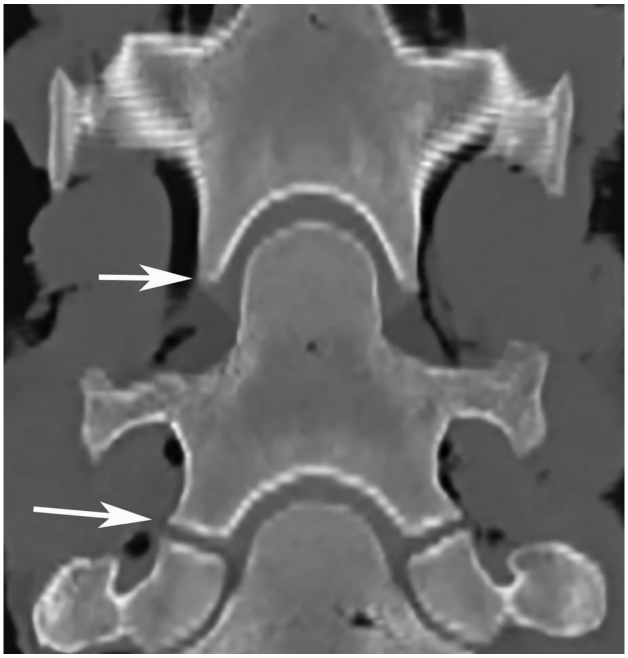

FIG. 5. Dorsal plane reconstructed CT image at the level of C7–Th1 and Th1–Th2, right side is at the left side; cranial is at the top of the image. No proper fovea costalis is present at the caudo-ventral aspect of the vertebral body of C7 due to the absence of ribs at Th1 (arrow). Note the appearance of a fovea costalis at the caudo-ventral aspect of Th1 bilaterally (arrow).

described or identified in horses. None of the horses in the present study had cervical ribs but 26 horses did present with a ventral protuberance, causing a change in position of the associated soft tissue structures and therefore possibly altered biomechanical function of this area.[11]

Homologous changes refer to morphological changes of size or shape of individual vertebrae.[5] Twenty-six of 78 horses (33.3%) had morphologic changes restricted to the transverse processes of C6 and C7 (otherwise named lateral vertebral or costal elements), described before as transposition of the caudal ventral tubercle from C6 to C7.[6,9] This is considerably more than the 13% or 24% prevalence described in the studies using conventional radiography and more in line with the findings during dissection of the thoroughbred-based population.[6,10] This difference is probably due to the higher sensitivity of CT compared to plain radiography, but may also have to do with differences in breed composition of the study populations. The relatively high prevalence in the present group of horses seems to plead for a rather benign character of these conformational changes in terms of clinical relevance. Although a cross-sectional observational study like ours cannot provide evidence about causal relationships, the fact that there was no significant difference in frequency between the groups of mature horses presented with or without clinical signs related to the cervical vertebral column (36.7% compared to 33.3%, $P = 0.81$) and foals (35.3% compared to 29.6%, $P = 0.72$) supports this conjecture to some extent. To define possible causal relationships of these homologous changes with clinical signs, a different subset of horses and standardized clinical examinations would be necessary.

The mammalian number of seven cervical vertebrae seems to have a very high degree of conservation. However, while maintaining this number, a shift in function and therefore related features of the last cervical and first thoracic vertebra has been described in giraffes as adaptations to biomechanical strain.[28] The variation in features of the transverse processes in C6 and C7 in the 26 horses presented in our study may be interpreted as a shift of function and features from C6 to C7 with alteration of biomechanical strain.[11] The assumption was that C7 takes on some of the features of C6 or "transposition of a ventral process from C6 to C7" seems likely, especially when considering embryogenesis.[6,9] During gastrulation of the mammalian embryo, somites are formed around the notochord. Each vertebra is formed by a caudal part of a cranial somite and a cranial part of a caudal somite, forming a sclerotome.[29] Hoxa-5 has been found to regulate the somites and thereafter sclerotomes in the cervical-thoracic transition area of chicken embryos.[30] Hoxa-5 contributes first to the regional vertebral patterning (homeotic), as it is expressed highly in the somites of this region. Thereafter, Hoxa-5 is expressed more specifically in the ventrolateral part of sclerotomes and plays a role in the formation of the lateral vertebral elements (costal elements) and the shaping of the morphology of the vertebra (homologous) after the determination of the vertebral pattern.[30] Although the vertebral patterning is predominantly maintained in the horses in our study, the lateral vertebral elements (transverse processes) were altered in 26 horses. A temporary and regional (lateroventral) deregulation of the Hoxa-5 gene in one somite could cause the loss of this element in C6 and presence in C7, when dividing in a cranial and caudal part. Therefore, the described changes seem to represent homologous variations in shape of these distal cervical vertebrae. The rest of the vertebral column was not examined in these horses and therefore no further differentiation can be made in axial homeotic or meristic variations.

In conclusion, findings indicated that familiarity with equine cervical vertebral anatomical variations during CT examinations is important, as homologous changes in this group of horses seem rather common. Homeotic variations in the equine occipitocervical or cervicothoracic vertebral junction in this group of horses were seen less commonly, but have to be considered as well. Although CT examination of the complete cervical spine in living horses became only recently possible, the CT images provided in this study can form a basis and reference for future studies as well as guidance in recognizing vertebral variations in other imaging modalities. Further work-up is warranted to identify the appearance and frequency of these changes in different imaging modalities such as radiography and ultrasonography. To identify possible clinical implications, different populations of horses with and without clinical signs attributable to this anatomic region, as identified with a standardized clinical examination, are needed.

LIST OF AUTHOR CONTRIBUTIONS

Category 1

(a) Conception and Design
Stefanie Veraa, A.J.M. Antoon-Jan van den Belt
(b) Acquisition of Data
Stefanie Veraa, A.J.M. Antoon-Jan van den Belt, Wilhelmina Bergmann, Inge Wijnberg, Willem Back
(c) Analysis and Interpretation of Data
Stefanie Veraa

Category 2

(a) Drafting the Article
Stefanie Veraa,
(b) Revising Article for Intellectual Content
Stefanie Veraa, Wilhelmina Bergmann, Inge Wijnberg, A.J.M. Antoon-Jan van de Belt, Willem Back

Category 3

(a) Final Approval of the Completed Article
Stefanie Veraa, Wilhelmina Bergmann, Inge Wijnberg, A.J.M. Antoon-Jan van de Belt, Willem Back

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