

Variation in the Seventh Lumbar Vertebra and the Lumbosacral Junction Morphometry Associated with the Sacrocaudal Fusion in Greyhounds

Sa'ad M.Y. Ismail¹ | Christina M. Murray¹ | Mark A. Stevenson¹ | Hung-Hsun Yen¹ | Helen, M.S. Davies¹

¹Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Victoria, 3010, Australia

Correspondence

Sa'ad M.Y. Ismail, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Victoria, 3010, Australia.
Email: saad.alzoubi@gmail.com

Funding

Melbourne International Research Scholarship (MIRS) from The University of Melbourne, Australia supported the study.

Abstract

Introduction: The lumbosacral joint is where the 7th lumbar vertebra (L.7) articulates within the sacrum. It is a clinically important area in the dog because of its relatively large range of motion. The current study aims to determine the possible differences in the length of the L.7 vertebra and the angle of the lumbosacral junction among greyhounds of standard and those of fused sacra, and to determine the potential association of sex, body mass, and type of fused sacrum (standard and fused) on the morphology of the L.7 vertebra and the angle of the lumbosacral junction.

Methods: Radiographs of 55 greyhound cadavers were used for radiographing, all radiographic images were stored and measured using X-ray acquisition software, and then analyzed using descriptive statistics, multiple linear regression, and logistic regression.

Results: The results of this study showed a significant increase ($P < 0.008$) in the length of the L.7 vertebra and the angle of the lumbosacral junction ($P < 0.028$) in greyhounds with fused sacra comparing with those of standard sacra, but the L.6 length was not significant ($P=0.431$).

Conclusion: Differences have been found in the length of L.7 vertebra and the angle

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/AHE.12675

of the lumbosacral junction in greyhounds. It was found that, in greyhounds, any variation in the sacrum's anatomical features may alter the structure of the surrounding anatomical structures such as the L.7 vertebra and lumbosacral junction.

KEYWORDS

Greyhounds, Seventh Lumbar Vertebra, Lumbosacral Junction, Sacrocaudal Fusion, Sacrum.

1 | INTRODUCTION

Understanding the biomechanics of the spine is important to understand normal locomotion, especially when considering the fact that different animal species vary in their vertebral column anatomy and related characteristics such as the vertebral shape and number. For example, the normal number of lumbar vertebrae in horses, oxen, and dogs is five to seven, six, and seven respectively (Evans & De Lahunta, 2013a; Liebich & König, 2010; Levine, Levine, Hoffman, Mez, & Bratton, 2007).

Any variation may create changes in the function of structures within or around the spine (Bürger & Lang, 1993). Similarly, the shape, orientation, and size of the joints between different bones can affect the function of the surrounding regions. The sacrum in dogs has four main joints; the lumbosacral, the sacrocaudal, and the paired (right and left) sacroiliac joints (Evans & De Lahunta, 2013a).

The seventh lumbar vertebra (L.7) has a body, spinous process, cranial and caudal articular surfaces, and paired costal processes (Figure 1). In dogs, there are seven lumbar vertebrae, which their bodies are generally longer than those vertebrae of the other regions. The width of the lumbar vertebrae from the 1st to the 7th usually increases as their length increases gradually from L.1 to L.5 or L.6 vertebra (Evans & De Lahunta, 2013a). Each lumbar vertebra has a spinous process, caudal and cranial articular surfaces, transverse processes, mammillary processes, caudal articular processes, and body (Evans & De Lahunta, 2013a). All lumbar vertebrae are characterized by their enlarged cranial and caudal end plates, except for the L.7, which has less enlarged end plate and articulates with the sacrum (Sisson, Grossman & Getty, 1975).

The lumbosacral joint is where the L.7 articulates with the sacrum (Evans & De Lahunta, 2013b) and this is clinically

important area in dogs because it may be involved in the lumbosacral transitional anomaly in many dog breeds. In dogs, it has been reported that the L.7 is shorter than the rest of the lumbar vertebrae (Hermanson & Lahunta, 2020; Miller, Evans, & Christensen, 1979). A lumbosacral transitional vertebra is a vertebra within vertebral column that might be shown to have the characteristics of both segments; lumbar and sacral (Larsen, 1977). A lumbosacral transitional vertebra is an anomalously formed vertebra involving both lumbar and sacral vertebra, which has been reported to affect up to 3.5 % of medium to large sized breed dogs with no relation to sex (Damur - Djuric, Steffen, Hässig, Morgan, & Flückiger, 2006; Flückiger, Steffen, Hässig, & Morgan, 2017; Morgan, 1999). German shepherd dogs with lumbosacral transitional vertebra have a longer L.7 in relation to the L.6 vertebra than dogs with normal lumbosacral junctions (Lappalainen, Salomaa, Junnila, Snellman, & Laitinen-Vapaavuori, 2012).

Two main forms of lumbosacral transitional vertebra have been reported in dogs; sacralisation and lumbarization. Sacralisation of the last lumbar vertebra (L.7) occurs when the L.7 attached (partially or completely) to the sacrum (Lappalainen *et al.*, 2012), and it has been reported to cause pressure on the nerve of the L.7 root in dogs such as in German Shepherds (Fearnside & Black, 2000). Lumbarization occurs when the S1 vertebra is partially or incompletely attached to its fused sacral component (S2) (Morgan, 1968).

The lumbosacral region is under complex physiological load (Benninger *et al.*, 2004) and the L7-S1 segment is under lower torsional stiffness compared to the rest of the lumbosacral region and this may explain the high range of spine motion at the level of the L7 and S1 vertebrae (Hediger *et al.*, 2009). An increase in the amount of spine flexion and extension in the lumbosacral region has been reported in large dogs (Benninger *et al.*, 2004). Such an increase in the range of the movement might be associated with the large forces applied on the bones. Variation in the anatomy and

morphology of the lumbosacral junction or its stability may have clinical significance (Schmid & Lang, 1993).

A recent study by Oheida, Philip, Yen & Davies (2016); on the prevalence of sacrocaudal fusion in greyhounds in Victoria, Australia reported that 41% of specimens had a sacrum containing 4 vertebrae.

This study aimed to determine the possible differences in the length of the L.7 and the angle of the lumbosacral junction between greyhounds with standard and those with fused sacra. Also, this study aimed to determine the potential association of sex, body mass, and type of fused sacrum (standard and fused) on the morphology of the L.7 and the angulation of the lumbosacral junction.

2 | MATERIALS AND METHODS

2.1. | Subjects

For this study, sacra of 55 greyhounds that have more than two years old and have no identifiable conditions related to the skeleton or vertebral columns, were used. The greyhounds used in this study were collected as cadavers and had been euthanized for reasons unrelated to this study.

2.2. | Radiographic measurements of L.6, L.7 vertebrae and angle of lumbosacral junction

For the radiograph study relating to L.6, L.7, and LSJ angel, only samples known sex were used (n=53). Greyhound cadavers were placed in right lateral recumbency, with the femur positioned perpendicular to the spine. For each greyhound, a set of two lateral radiographs of the lumbosacral region was taken using a portable radiographic machine (Atomscope HF80/15 UltraLight, Mikasa X-ray Co Ltd, Japan). Each cadaver was exposed to a peak kilovoltage of 80 kVp, an exposure time of 0.18 seconds, and 2.7 milliamp seconds (mAs). The X-ray collimator was attached to a portable stand parallel to the ground at a fixed distance of one meter from the cassette.

All radiographic images were processed by a Veterinary-80 CR Scanner (3D Imaging & Simulations Corp., Yuseong-Gu, Daejeon, Korea). Images were stored, analysed, and measured with X-ray acquisition software (dicom PACS® DX-R). Measurements and data recording the lengths of the 6th vertebra (L.6), L.7, and the angle of the lumbosacral junction for each greyhound were

performed twice by a researcher and then repeated by another. The lengths of the L.6 and the L.7 were recorded by taking the midcorpus length in mm (half way between the dorsal and ventral border of the vertebral body) as described in other studies (Di Concetto, Mandsager, Riebold, Stieger-Vanegas, & Killos, 2012; Lappalainen *et al.*, 2012) (Figure 2). The angle of the lumbosacral junction is the angle formed by two lines bisecting the vertebral body of the L.7 and the sacrum as described in a previous study (Schmid & Lang, 1993) (Figure 3).

2.3. | Bone specimen collection

Cadavers were bisected transversely at the level of the 6th lumbar vertebra (L.6) using an industrial meat bandsaw (Thompson machinery: Circa 1965, Queensland, Australia) to obtain the caudal part of the body containing the L.7, sacrum. After removing the most of the soft tissue from the collected specimens, the bones (sacrum and L.7) were boiled for 48 hours at 98.5 °C using a steam-water boiler (A.E Athena Equipment, manufactured by Atherton & Sons Pty Ltd) to remove the remaining soft tissues. The sacra were then dried using a Brown built deluxe upright dryer for 8 hours before the measurements start.

2.4. | Classification of sacra

Fifty five Sacra were classified into standard (consisted of three fused vertebrae) and fused (consisted of four fused vertebrae) as described by Oheida et al. (2016).

2.5. | Statistical analyses

Data was analyzed using the SPSS statistical package (IBM, SPSS version 23, 2013). Intra-rater and inter-rater reliability (test-retest reliability) was assessed using Lin's concordance correlation coefficient (Lawrence & Lin, 1989; Steichen & Cox, 2002). Concordance correlation coefficient values range from -1 to +1 (+1 indicating strong positive agreement and -1 indicating strong negative agreement). For continuous nominal (categorical) variables, the Kappa statistics test was used and the strength of agreement evaluated in accordance to Landis & Koch, (1977) as flowing; poor agreement if the Kappa value was less than zero, a slight agreement if the Kappa range was between 0.00 - 0.20, fair agreement when Kappa is between 0.21-0.40, moderate when Kappa is between 0.41-0.60, substantial when Kappa is between

0.61-0.80, and almost perfect agreement when Kappa is 0.81-1.00.

The descriptive statistics for the measurements of L.6 and L.7 vertebrae and the angle of the lumbosacral junction of greyhounds stratified by sacrum classification are provided (Table 5). Measurement data was plotted as frequency histograms to confirm that it follows normal distribution. The normality of each plotted distribution was assessed using the Shapiro-Wilk test. The Shapiro-Wilk test was used in preference to the Kolmogorov-Smirnov test because the number of measurements in this study was relatively small ($n = 53$). The equality of the variances for each of the measurements for each of the sacrum types was assessed using Levene's test.

A multiple linear regression analysis was used to quantify the association between the measurements of L.6 and L.7 vertebrae, angle of the LSJ and sacral type (standard and fused), and body mass and sex (as explanatory variables). Multiple linear regressions allowed us to provide estimates of measurements for the two sacral types, adjusting for the confounding effects of body mass and sex. Our linear regression model took the form:

$$L.7 \text{ Length}_i = \beta_0 + \beta_1 \text{type}_i + \beta_2 \text{sex}_i + \beta_3 \text{body mass}_i \quad (1)$$

In Equation (1), $L.7 \text{ length}_i$ represents the length of the L.7 for the i^{th} greyhound, β_0 is the intercept term, β_1 is the regression coefficient for sacrum type (a categorical variable comprised of two levels; fused and standard), β_2 is the regression coefficient for sex (a categorical variable comprised of two levels; male and female), and β_3 is the regression coefficient for body mass. Similar linear regression models were developed for the L.6 vertebra length and the angle of LSJ.

Logistic regression analysis was used to quantify the association between the length of the L.7 (as the outcome variable) and body mass and sex (as explanatory variables). This allowed us to estimate the association between sex and the length of the L.7, adjusting for the confounding effect of body mass. Our logistic regression model took the form:

$$\text{logit}(p_i) = \beta_0 + \beta_1 \text{sex}_i + \beta_2 \text{body mass}_i \quad (2)$$

In Equation (2); $\text{logit}(p_i)$ represents the logit of the probability of i^{th} greyhound having a fused sacrum, β_0 is the intercept term, β_1 is the regression coefficient for sex (a categorical variable comprised of two levels; male and female), and β_2 is the regression coefficient for body mass. In all analyses, a P-value of < 0.05 is considered statistically significant.

3 | RESULTS

3.1. | Anatomy of the L.7 vertebra in greyhounds

In the greyhound, the 7th lumbar vertebra consists of a body, spinous process, and a pair of costal processes directed cranially. There are caudal and cranial articular processes with mammillary process on the cranial one. (Figure 4). Apart from the length of the body, no other anatomical differences were noticed between the L.7 in greyhounds with standard sacra and those with sacrocaudal fusion.

3.2. | Reproducibility/ repeatability of data

Intra-rater and inter-rater reliability (test-retest reliability) were assessed and showed a high level of concordance between the 1st and 2nd readings of the same investigator (Table 1); and between the readings of the first investigator and the second investigator (Table 2).

The inter-rater reliability (test-retest reliability) was assessed and showed almost perfect agreement between the classifications of the first and second investigators; Kappa = 0.89 ($P < 0.000$) (Landis & Koch, 1977). (Table 3).

3.3. | Prevalence of sacrocaudal fusion and measurements of the L.6 and L.7 vertebrae and angle of lumbosacral junction

Among the 55 cadavers, the prevalence of sacrocaudal fusion across all males and females greyhounds in this study was 39.6% ($n=53$). Among the 53 greyhound cadavers, 26 cadavers were those of males (49.1%) and 27 were those of females (50.9%) (Table 4).

Using radiographs enabled us to confirm the occurrence of sacrocaudal fusion and distinguish fused sacra ($n= 22$) from standard ones ($n=33$). However, for the radiograph study, only samples known sex were used ($n=53$). It was not difficult to see that the standard sacrum was characterized by the presence of space between the S3 and Ca1 vertebral bodies in lateral radiographs. The descriptive statistics of each sacral measurements stratified by sacral classification for all greyhounds

under this study were combined and presented in Table 5. The highest values were for the fused sacra.

3.4. | The association between the type of sacrum, sex and body mass and the morphology of L.6 and L.7 vertebrae and the angle of LSJ

The estimated regression coefficients of the linear regression model and related standard errors for the association between type of sacrum, sex, and body mass and length of L.6 and L.7 vertebrae and the angle of the lumbosacral junction are shown in Tables (6), (7), and (8) respectively. After adjusting for the effect of sex and body mass, the length of L.7 in greyhounds with fused sacra was 1.54 mm (95% CI -2.67 to -0.42, $P < 0.008$) longer than those in the standard sacra (Table 6). After adjusting for the effect of type of sacrum and body mass, the length of L.7 in male greyhounds was not significantly longer than those in female greyhounds ($P = 0.31$). One-kilogram increase in body mass was associated with a 0.11 mm (95% CI -0.13 to 0.34, $P = 0.37$) increase in the length of L.7 (mm)..

After adjusting for the effect of sex and body mass, the length of L.6 in greyhounds with fused sacra was not significantly longer ($P = 0.431$) than those in fused sacra (Tables 7). After adjusting for the effect of type of sacrum and body mass, the length of L.6 vertebra in male greyhounds was not significantly longer ($P < 0.052$) than those in female greyhounds. One-kilogram increase in body mass was associated with a 0.13 mm (95% CI -0.44 to 0.312, $P = 0.137$) increase in the length of the L.6 vertebra (mm).

After adjusting for the effect of sex and body mass, the angle of the lumbosacral junction in greyhounds with fused sacra was significantly 3.18 degrees (95% CI -6 to -0.358, $P < 0.028$) higher than those in fused sacra (Tables 8). After adjusting for the effect of type of sacrum and body mass, the angle of the lumbosacral junction in male greyhounds was not significantly different ($P = 0.285$) to those in females. One-kilogram increase in body mass was associated with a 0.07 degree (95% CI -0.52 to 0.66, $P = 0.808$) increase in the angle of the lumbosacral junction (°).

3.5. | Length of the L.7 vertebra, sex and the prevalence of sacrocaudal fusion in greyhounds

The estimated regression coefficients of the logistic regression model and related standard errors for the association between sex

and body mass on the length of L.7 (presence of fused sacra) are shown in Table (9).

After adjusting for the effect of body mass, there were no statistically significant associations identified between sex and length of the L.7 ($z = 0.089$; $P = 0.765$). Also, after adjusting for the effect of sex, there were no statistically significant ($z = 0.063$; $P = 0.802$) associations identified between body mass (kg) and the length of L.7 (mm).

4 | DISCUSSION

Little attention has been paid to the anatomy of the L.7 in greyhounds. Direct visual observations showed that the shape and general anatomy of the L.7 in greyhounds with fused sacra were the same as those with standard sacra (Figure 4) and similar to the description of canine anatomy of the L.7 in the literature (Evans & De Lahunta, 2013b; Goody, 1997).

Investigating the radiographs we showed a space between the sacrum and first caudal vertebra in greyhounds with standard sacrum, however this space almost disappeared in those greyhounds with sacrocaudal fusion (figure 2). In this study, radiographs were used to classify sacra into two types; standard and fused based on the space between S3 and Cd1 vertebrae (Figure 2), and this classification agreed with the classification of sacra after collecting the bones from cadavers and cleaning them, which validates the use of radiographs for sacra classifying by the number of fused vertebrae, (Table 3). In the previous study by Oheida *et al.* (2016), the correlation between classifying sacra through the radiographs of bones and classifying sacra after collecting them was very high, this supports the findings of this study (Oheida *et al.*, 2016). However, it was not easy to use radiographs to distinguish the different types of fused sacra.

The results of this study showed a significant increase in the length of the L.7 and the angle of the lumbosacral junction in greyhounds with fused sacra comparing to standard sacra. This increase in the length of L.7 might enhance the role of this vertebra in the passive system of force transition between vertebrae (Moens & Runyon, 2002), which may increase the efficiency of force transition within the spine. The length of the L.7 and the angle of the lumbosacral junction were both influenced by the occurrence of sacrocaudal fusion. Supported the hypothesis that there are measurable differences in the length of the L.7 and the angle of the lumbosacral junction between greyhounds with standard and fused sacra (Tables (6) and (8)). Also, the length of the L.7 and the angle of the lumbosacral junction was not significantly influenced by sex

or body mass of the greyhounds. However, body mass and sex tend to have an association with the length of the L.6 and L.7, and the angle of the lumbosacral junction. Table (8).

Another interesting finding was that the sacrocaudal fusion significantly influenced the length of the L.7 while it also tended to increase the length of the L.6 vertebra but not significantly. This relative difference of the effects of sacrocaudal fusion between L.7 and L.6 vertebrae may be because of the direct articulation between the sacrum and L.7, but in general, it still shows the potential of sacrocaudal fusion to influence more structures surrounding the sacral region.

This variation in the lumbosacral junction between greyhounds with standard and fused sacra may have a clinical significance. The normal shape of the intervertebral disc of L7-S1 from the lateral view is a triangle (Meij & Bergknut, 2010). The intervertebral disc is the most important structure within the spine that maintains the stability between all vertebrae (Adams & Roughley, 2006; Krismer, Haid, Ogon, Behensky, & Wimmer, 1997; Zhao, Pollintine, Hole, Dolan, & Adams, 2005). The increase in the angle of the lumbosacral junction made the end plates of both L.7 and S1 vertebrae to be almost parallel and thus widened the junction dorsally. In fact, the lumbosacral junction is important because it is the site for epidural analgesic drug injections for many clinical and surgical procedures (Di Concetto *et al.*, 2012) and has been selected for this purpose because it is easy to be located (Campoy, 2004; Halley & Riedesel, 1983). For this reason, our result suggests that an increase in the angle of the lumbosacral junction by the occurrence of sacrocaudal fusion may enhance the ease of lumbosacral epidural injections in greyhounds..

The findings of this study may have an impact on the research area of the spine, especially for the sacral region. The increase in the length of the L.7 and the angle of the lumbosacral junction may alter the stability of this segment of the spine, which could lead to many complications. It has been reported that the instability of this spinal segment might shift the load-bearing from the intervertebral disc to other parts of the spine such as ventral aspects of vertebral bodies and the articular facet joints (Meij & Bergknut, 2010) and any abnormal motion within the lumbosacral junction would cause a degeneration of the disc between L.7 and S1 vertebrae (Adams & Roughley, 2006; Kaigle, Holm, & Hansson, 1995; Tanaka *et al.*, 2001). The findings of this study are very important because it has been reported that the motion (flexion-extension and lateral bending) within the vertebral column is the highest within the lumbosacral junction, followed by the L.5- L.6 segment during the extension and the flexion of the spine (Braund, Taylor, Ghosh, & Sherwood, 1977; Bürger & Lang, 1993). The increase in the length

of L.6 and L.7 vertebrae, and the angle of the lumbosacral junction in association with sacrocaudal fusion, may alter the extension and the flexion of the spine at this lumbosacral region, which needs to be investigated in the future.

In dogs with certain complications such as degenerative lumbosacral stenosis, the flexion-extension pattern motion of the lumbosacral segment is found to be reduced comparing with healthy dogs (Schmid & Lang, 1993). Also, it has been found that dogs with abnormal lumbosacral junctions have different motion patterns comparing with healthy dogs (Gradner, Bockstahler, Peham, Henninger, & Podbregar, 2007).

The variations in the angle of the lumbosacral junction have been used to differentiate between normal dogs and those with cauda equina syndrome (Schmid & Lang, 1993), therefore, it could be an advantage for greyhounds or a clinical sign of certain complications, and these need to be investigated, too. Comparing the angle of the lumbosacral junction between greyhounds with standard and fused sacra showed a significant increase in those with fused sacra and this means that the V shape of the disc space will open dorsally with the remaining space ventrally open, too, which agrees with the finding of Morgan and Bailey (1990) on their study on German Shepherd dogs, making the endplates of L.7 and S1 vertebrae more parallel. The increase in the angle of the lumbosacral joint changed the shape of the lumbosacral space (Morgan, & Bailey, 1990). This variation in the angle may be related to the reduction in the flexibility of the lumbosacral junction in association with fusion. The decrease in the angle of the lumbosacral joint in females comparing with males suggests that those females have a more lordotic posture, as it is also suggested for German Shepherds and Labrador retrievers (Schmid & Lang, 1993). It remains to be investigated whether the increase in the length of L.6 and L.7 vertebrae, and the angle of the lumbosacral joint can be related to the locomotion of greyhounds or specific diseases such as cauda equina syndrome or disc degeneration.

It seems there is a clinical relevance in the increase of the length of L.7 in association with sacrocaudal fusion in greyhounds. In humans, it has been reported that the fusion between L.5 and the sacrum might cause an advanced degeneration in the intervertebral disc (Edwards *et al.*, 2004). The increase in the length of L.7 may decrease the space between the L.7 and the sacrum causing more pressure and thus affects the intervertebral disc, which may lead to similar clinical signs to those of cauda equina syndrome, such as degenerative lumbosacral stenosis. One of the main functions of the intervertebral disc is to absorb force and stabilize the vertebrae (Chan, Au, Tam, Cheah, & Chan, 2014). In fact, degeneration of the intervertebral disc, which is common in

canine breeds, classified as chondrodysplasia (Hansen, 1951). A narrowing in the intervertebral discs between the thoracic vertebrae has been noticed in association with the thoracic vertebral fusion in Beagle puppies (Coleman, 1968).

Indeed, some variations in the morphology of the L.7 were expected to be found since it is the first bone articulating within the sacrum cranially and might suppose to reflect the influence of sacrocaudal fusion, if any exists. This increase in the length of L.7 and sacrum (length of the sacrum increased as a result of the fusion between the S3 and Ca1 vertebrae), in association with sacrocaudal fusion, might have clinical implications. An example of these implications is a reduction in the intervertebral space between the sacrum S1 and L.7 vertebrae, as a result of the increase in the sacrum and L.7 length, which might affect the lumbosacral joint function. In addition, fused sacra tend to be stiffer and more concave ventrally (increase in curvature of the pelvic surface), which tilts the sacrum cranially and ventrally. This might also reduce the intervertebral space between the sacrum and the caudal end plate of the L.7.

The results of this study highlight the need of finding the relation between the occurrence of sacrocaudal fusion reduction in the vertebral bodies and cauda equine syndrome. Moreover, the findings of this study suggest that any variations in the sacrum's anatomical features might alter the function of the sacrum and the nearby anatomical structures. Clinical diagnosis should consider the occurrence of sacrocaudal fusion before making a final diagnosis. Finally, the results of this study support our hypothesis that the occurrence of sacrocaudal fusion may favour a change in the morphology of L.7 in greyhounds.

The limitations of our study were the lack of radiographs of the whole vertebral column, the low number of greyhounds used in this study, and the age of the greyhounds under study, however, the results were statistically significant.

5 | CONCLUSION

In this study, it has been found that the occurrence of sacrocaudal fusion in greyhounds has significantly increased the length of the L.7 and the angle of the lumbosacral junction. Besides, the length of the L.7 has significantly increased with sacrocaudal fusion, which has been found to increase the length and width of the sacrum, and that might affect the alignment and biomechanical properties of the lumbosacral segment and the intervertebral disc. It can be concluded that any variations in the sacrum's anatomical features may alter the function of the sacrum

and the surrounding anatomical structures, such as L.7. Clinical reports and diagnosis should consider the presence or absence of sacrocaudal fusion in greyhounds, before making a final diagnosis of any related clinical complications. Finally, the radiograph of the sacral region has the potential to successfully evaluate and classify the types of sacra into two types; standard or fused.

Acknowledgement

The study was supported by Melbourne International Research Scholarship (MIRS) from The University of Melbourne, Australia. The authors would like to thank Brendan Kehoe and Harshanie Abeywardena for assistance with samples collection and storage. Also, Cameron Patrick of the Melbourne Statistical Consulting Platform for statistical assistance.

Conflict of Interest

No conflict of interest is declared for this work.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon request.

REFERENCES

- Adams, M. A., & Roughley, P. J. "What is intervertebral disc degeneration, and what causes it?." *Spine* , 31.18 (2006): 2151-2161. doi.org/10.1097/01.brs.0000231761.73859.2c.
 - Benninger, M. I., Seiler, G. S., Robinson, L. E., Ferguson, S. J., Bonél, H. M., Busato, A. R., & Lang, J.(2004). Three-dimensional motion pattern of the caudal lumbar and lumbosacral portions of the vertebral column of dogs. *American journal of veterinary research*, 65, 544-551. doi.org/10.2460/ajvr.2004.65.544.
 - Braund, K. G., Taylor, T. K. F., Ghosh, P., & Sherwood, A. A. (1977). Spinal mobility in the dog. A study in chondrodystrophoid and non-chondrodystrophoid animals. *Research in Veterinary Science*, 22, 78-82. doi.org/10.1016/S0034-5288(18)33317-4.
 - Bürger, R., & Lang, J. (1993). Kinetic studies of the lumbar vertebrae and the lumbosacral transition in the German shepherd dog. 2. Our personal investigations. *Schweizer Archiv für Tierheilkunde*, 135, 35-43.
- from: <https://europepmc.org/article/med/8456269>.

- Campoy, L. (2004). Epidural and spinal anaesthesia in the dog. *In Practice*, 26, 262-269. doi.org/10.1136/inpract.26.5.262.
- Chan, W. C., Au, T. Y., Tam, V., Cheah, K. S., & Chan, D. (2014). Coming together is a beginning: the making of an intervertebral disc. *Birth Defects Research Part C: Embryo Today: Reviews*, 102, 83-100. doi.org/10.1002/bdrc.21061.
- Coleman, S. S. (1968). The effect of posterior spine fusion on vertebral growth in dogs. *The Journal of Bone & Joint Surgery*, 50, 879-896. doi.org/10.2106/00004623-196850050-00002.
- Damur-Djuric, N., Steffen, F., Hässig, M., Morgan, J. P., & Flückiger, M. A. (2006). Lumbosacral transitional vertebrae in dogs: classification, prevalence, and association with sacroiliac morphology. *Veterinary radiology & ultrasound*, 47, 32-38. doi.org/10.1111/j.1740-8261.2005.00102.x.
- Di Concetto, S., Mandsager, R. E., Riebold, T. W., Stieger-Vanegas, S. M., & Killos, M. (2012). Effect of hind limb position on the craniocaudal length of the lumbosacral space in anesthetized dogs. *Veterinary Anaesthesia and Analgesia*, 39, 99-105. doi.org/10.1111/j.1467-2995.2011.00676.x.
- Edwards, C. C., Bridwell, K. H., Patel, A., Rinella, A. S., Berra, A., & Lenke, L. G. (2004). Long adult deformity fusions to L5 and the sacrum a matched cohort analysis. *Spine*, 29, 1996-2005. doi.org/10.1097/01.brs.0000138272.54896.33.
- Evans, H. E., & De Lahunta, A. (2013a). *Miller's Anatomy of the Dog*: 4th Edition, Elsevier Health Sciences.US.
- Evans, H. E., & De Lahunta, A. (2013b). *Miller's anatomy of the dog-E-Book*: 4th Edition Elsevier Health Sciences. US.
- Fearnside, S., & Black, A. P. (2000). The use of MRI to diagnose a lateralising disc extrusion in a dog with a transitional vertebra. *Australian Veterinary Practitioner*, 30, 98-102. doi/abs/10.1111/j.1751-0813.1971.tb15498.x
- Flückiger, M. A., Steffen, F., Hässig, M., & Morgan, J. P. (2017). Asymmetrical lumbosacral transitional vertebrae in dogs may promote asymmetrical hip joint development. *Veterinary and Comparative Orthopaedics and Traumatology*, 30, 137-142. From www.vcot-online.com on 2017-03-20 | ID: 1000489118 | IP: 79.98.0.37.
- Goody, P. C. (1997). *Dog anatomy: a pictorial approach to canine structure*. J. A. Allen & Co. Ltd. Publisher. UK.
- Gradner, G., Bockstahler, B., Peham, C., Henninger, W., & Podbregar, I. (2007). Kinematic study of back movement in clinically sound malinois dogs with consideration of the effect of radiographic changes in the lumbosacral junction. *Veterinary Surgery*, 36, 472-481. doi.org/10.1111/j.1532-950X.2007.00294.x
- Halley, Lynn E. and Riedesel, Dean H. (1983) "Epidural Analgesia in the Dog," *Iowa State University Veterinarian*: Vol. 45 : Iss. 1 , Article 9. From: https://lib.dr.iastate.edu/iowastate_veterinarian/vol45/iss1/9
- Hansen, H. J. (1951). A pathologic-anatomical interpretation of disc degeneration in dogs. *Acta Orthopaedica Scandinavica*, 20, 280-293. doi.org/10.3109/17453675108991175
- Hediger, K. U., Ferguson, S. J., Gedet, P., Busato, A., Forterre, F., Isler, S., ... & Lang, J. (2009). Biomechanical analysis of torsion and shear forces in lumbar and lumbosacral spine segments of no chondrodystrophic dogs. *Veterinary Surgery*, 38, 874-880. doi.org/10.1111/j.1532-950X.2009.00582.x
- Hermanson, J. W., & Lahunta, A. D. (2020). *Miller's anatomy of the dog. Miller's anatomy of the dog*: 5th Edition., (Ed. 5). Elsevier Inc. USA.
- Kaigle, A. M., Holm, S. H., & Hansson, T. H. (1995). Experimental instability in the lumbar spine. *Spine*, 20, 421-430. doi.org/10.1097/00007632-199502001-00004.
- Krismer, M., Haid, C., Ogon, M., Behensky, H., & Wimmer, C. (1997). Biomechanics of lumbar instability. *Der Orthopäde*, 26, 516-520. doi.org/10.1007/PL00003406.
- Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics*, 159-174. doi.org/10.2307/2529310
- Lappalainen, A. K., Salomaa, R., Junnila, J., Snellman, M., & Laitinen-Vapaavuori, O. (2012). Alternative classification and screening protocol for transitional lumbosacral vertebra in German shepherd dogs. *Acta Veterinaria Scandinavica*, 54, 27. doi.org/10.1186/1751-0147-54-27
- Larsen, J. S. (1977). Lumbosacral transitional vertebrae in the dog. *Veterinary Radiology & Ultrasound*, 18, 76-79. doi.org/10.1111/j.1740-8261.1977.tb01126.x.
- Lawrence, I., & Lin, K. (1989). A concordance correlation coefficient to evaluate reproducibility. *Biometrics*, 255-268. from: http://www.jstor.org/stable/2532051.
- Levine, J. M., Levine, G. J., Hoffman, A. G., Mez, J., & Bratton, G. R. (2007). Comparative anatomy of the horse, ox, and dog: the vertebral column and peripheral nerves. *Equine compendium on continuing education for the practicing*

- veterinarian*, 2, 279-292. from:
http://assets.prod.vetlearn.com.s3.amazonaws.com/mmah/c6/5837c24b2042b3ab205b5fdd405101/filePVE_02_09_27_9_0.pdf.
- Liebich, H. G. and König, H. E. (2014). *Veterinary anatomy of domestic mammals: textbook and colour atlas* 4th Edition, Schattauer Verlag, Germany.
- Meij, B. P., & Bergknut, N. (2010). Degenerative lumbosacral stenosis in dogs. *Veterinary Clinics: Small Animal Practice*, 40, 983-1009.
doi.org/10.1016/j.cvsml.2010.05.006.
- Miller, M. E., Evans, H. E., & Christensen, G. C. (1979). *Miller's Anatomy of the Dog*. 5th Edition. Elsevier Inc. USA.
- Moen, N. M., & Runyon, C. L. (2002). Fracture of L7 vertebral articular facets and pedicles following dorsal laminectomy in a dog. *Journal of the American Veterinary Medical Association*, 221, 807-810.
doi.org/10.2460/javma.2002.221.807.
- Morgan, J. P. (1968). Congenital anomalies of the vertebral column of the dog: a study of the incidence and significance based on a radiographic and morphologic Study 1. *Veterinary Radiology*, 9, 21-29. doi.org/10.1111/j.1740-8261.1968.tb01082.x.
- Morgan, J. P. (1999). Transitional lumbosacral vertebral anomaly in the dog: a radiographic study. *Journal of small animal practice*, 40, 167-172.
doi.org/10.1111/j.1748-5827.1999.tb03784.x.
- Morgan, J. P., & Bailey, C. S. (1990). Cauda equina syndrome in the dog: radiographic evaluation. *Journal of Small Animal Practice*, 31, 69-77. doi.org/10.1111/j.1748-5827.1990.tb00724.x.
- Oheida, A. H., Philip, C. J., Yen, H. H., & Davies, H. M. (2016). Observations of sacrocaudal fusion in Greyhounds and other dogs. *Veterinary and Comparative Orthopaedics and Traumatology*, 29, 61-67. doi.org/10.3415/VCOT-15-04-0069.
- Schmid, V., & Lang, J. (1993). Measurements on the lumbosacral junction in normal dogs and those with cauda equine compression. *Journal of Small Animal Practice*, 34, 437-442. doi.org/10.1111/j.1748-5827.1993.tb03897.
- Sisson, S., Grossman, J. D. & Getty, R. (1975). *The Anatomy of the Domestic Animals*, 5th edition, W B Saunders Co, Philadelphia, Pennsylvania.
- Steichen, T. J., & Cox, N. J. (2002). A note on the concordance correlation coefficient. *The Stata Journal*, 2, 183-189.
doi.org/10.1177/1536867X0200200206.
- Tanaka, N., An, H. S., Lim, T. H., Fujiwara, A., Jeon, C. H., & Haughton, V. M. (2001). The relationship between disc degeneration and flexibility of the lumbar spine. *The spine journal*, 1(1), 47-56. doi.org/10.1016/S1529-9430(01)00006-7.
- Zhao, F., Pollintine, P., Hole, B. D., Dolan, P., & Adams, M. A. (2005). Discogenic origins of spinal instability. *Spine*, 30, 2621-2630. doi.org/10.1097/01.brs.0000188203.71182.c0.

Figure Legends:

FIGURE 1 Anatomy of the 7th lumbar vertebra (L.7) in the greyhound. Author's own.

FIGURE 2 Measurements were recorded by taking the midcorpus (half way between the dorsal and ventral border of the vertebral body) length (mm) of the L.6 and L.7 vertebrae in greyhounds with standard sacrum (Top image shows joint space between S3 and Cd1 vertebrae) and those with sacrocaudal fusion (Bottom image). L.6: sixth lumbar vertebra L.7: seventh lumbar vertebra. Cd1: 1st caudal vertebra. Author's own.

FIGURE 3 Radiograph showing how the measurements of the angle of the lumbosacral junction were taken. Angle (*) was formed by the intersection of two lines that bisect the midcorpus of the sacrum and L.7 vertebra. L.6: sixth lumbar vertebra L.7: seventh lumbar vertebra. Cd1: 1st caudal vertebra. Author's own.

FIGURE 4 Lateral aspect of the 7th lumbar vertebra (L.7) in greyhounds with fused sacrum. Author's own.

Table Legends

TABLE 1 Intra-rater correlations of measurements (L.7, L.6, and Angle LSJ) of greyhounds.

TABLE 2 Inter-rater correlations of measurements (L.7, L.6, and Angle LSJ) of greyhounds.

TABLE 3 Interrater Reliability test for type of sacrum (standard or fused) using Kappa test.

TABLE 4 Contingency table showing the frequency of standard and fused sacra, by sex in greyhounds.

TABLE 5 Descriptive statistics of each of the sacral measurements described in this study, stratified by sacral classification (standard and fused).

TABLE 6 Regression coefficients and their standard errors from a linear regression model of factors influencing the length of 7th lumbar vertebra (L.7 (mm)) in greyhounds.

TABLE 7 Regression coefficients and their standard errors from a linear regression model of factors influencing the length of 6th lumbar vertebra (L.6 (mm)) in greyhounds.

TABLE 8 Regression coefficients and their standard errors from a linear regression model of factors influencing the angle of lumbosacral junction (LSJ°) in greyhounds.

TABLE 9 Regression coefficients and their standard errors from a logistic regression model of factors influencing the length of the L.7 vertebra (presence of fused sacra) in greyhounds.

Author Manuscript

TABLES

Table 1. Intra-rater correlations of measurements (L.7, L.6, and Angle LSJ) of greyhounds.

| Measurement | CCC | P-value | 95% C.I |
|-------------|------|---------|--------------|
| L.7 (mm) | 0.96 | <0.001* | 0.93 to 0.98 |
| L.6 (mm) | 0.96 | <0.001* | 0.93 to 0.98 |
| Angle LSJ ° | 0.97 | <0.001* | 0.96 to 0.99 |

C.I.: Confidence Interval; CCC: Concordance correlation coefficient; L.6 and L.7: lumbar vertebra number six and seventh respectively; *: statistically significant as $P < 0.05$.

Table 2. Inter-rater correlations of measurements (L.7, L.6, and Angle LSJ) of greyhounds.

| Measurement | CCC | P-value | 95% C.I |
|-------------|------|---------|--------------|
| L.7 (mm) | 0.95 | <0.001* | 0.92 to 0.97 |
| L.6 (mm) | 0.98 | <0.001* | 0.97 to 0.99 |
| Angle LSJ° | 0.96 | <0.001* | 0.93 to 0.98 |

C.I.: Confidence Interval; CCC: Concordance Correlation Coefficient; L.6 and L.7: lumbar vertebra number six and seventh respectively; LSJ °: angle of lumbosacral junction; *: statistically significant as $P < 0.05$.

Table 3. Interrater Reliability test for type of sacrum (standard or fused) using Kappa test.

| Classification | | 2nd Reading | | Kappa value | P-value |
|-------------------------|----------|-------------|-----------|-------------|---------|
| | | Standard | Fused | | |
| | | N (%) | N (%) | | |
| 1 st Reading | Standard | 31 (93.9) | 2 (6.1) | 0.89 | <0.001* |
| | Fused | 1 (4.5) | 21 (95.5) | | |
| | Total | 32 (58.2) | 23 (41.8) | | |

LSJ°: angle of lumbosacral junction; *: statistically significant as $P < 0.05$

Table 4. Contingency table showing the frequency of standard and fused sacra, by sex in greyhounds.

| Type of Sacrum | Standard | Fused | Total |
|----------------|-----------|-----------|-----------|
| Sex | n (%) | n (%) | n (%) |
| Male | 16 (30.2) | 10 (18.9) | 26 (49.1) |
| Female | 16 (30.2) | 11 (20.8) | 27 (50.9) |
| Total | 32 (60.4) | 21 (39.6) | 53 (100) |

n: sample size.

Table 5. Descriptive statistics of each of the sacral measurements described in this study, stratified by sacral classification (standard and fused).

| Type of Sacrum | Measurement | n | Mean \pm SD | Median (Q1, Q3) | Min-Max |
|----------------|---------------|----|------------------|-----------------------|--------------|
| Standard | L.7 (mm) | 33 | 29.3 \pm 2.04 | 30.1 (28.0, 31.0) | 24.4-32.0 |
| | L.6 (mm) | 33 | 34.6 \pm 1.88 | 34.9 (33.3, 35.8) | 30.4- 38.4 |
| | Angel (LSJ) ° | 33 | 163.7 \pm 5.44 | 164.15 (158.6, 168.3) | 151.9- 173.2 |
| Fused | L.7 (mm) | 22 | 30.98 \pm 2.16 | 31.02 (29.29, 32.4) | 27.5- 34.7 |
| | L6 (mm) | 22 | 35.09 \pm 1.70 | 34.9 (33.7, 36.2) | 32.6- 39.0 |
| | Angel (LSJ) ° | 22 | 166.6 \pm 4.13 | 166.9 (163.1, 170.0) | 157.2- 173.0 |
| Total | L.7 (mm) | 55 | 30 \pm 2.22 | 30.2 (28.6, 31.4) | 24.4- 34.7 |
| | L.6 (mm) | 55 | 34.8 \pm 1.81 | 34.9 (33.5, 35.9) | 30.4- 39.0 |
| | Angel (LSJ) ° | 55 | 164.9 \pm 5.12 | 165.5 (162.1, 169.2) | 151.9- 173.2 |

n= sample size; SD: Standard deviation.

Table 6. Regression coefficients and their standard errors from a linear regression model of factors influencing the length of 7th lumbar vertebra (L.7 (mm)) in greyhounds.

| Explanatory Variable | Coefficient (SE) | t | P- value | 95% CI |
|----------------------|------------------|-------|----------|----------------|
| Intercept | 27.44 (3.25) | 8.43 | <0.01* | |
| Type of Sacrum: | | | | |
| Fused | Reference | | | - |
| Standard | -1.54 (0.56) | -2.76 | <0.008* | -2.67 to -0.42 |
| Sex: | | | | |
| Female | Reference | | | - |
| Male | 0.82 (0.79) | 1.03 | 0.31 | -0.78 to 2.4 |
| Body Mass (kg): | 0.11 (0.12) | 0.90 | 0.37 | -0.13 to 0.34 |

SE: Standard error; CI: Confidence Interval; *: statistically significant as P < 0.05.

Table 7. Regression coefficients and their standard errors from a linear regression model of factors influencing the length of 6th lumbar vertebra (L.6 (mm)) in greyhounds. n=53.

| Explanatory Variable | Coefficient (SE) | t | P- value | 95% CI |
|----------------------|------------------|-------|----------|----------------|
| Intercept | 30.5 (2.45) | 12.43 | <0.01* | |
| Type of Sacrum: | | | | |
| Fused | Reference | | | - |
| Standard | -0.34 (0.42) | -0.79 | 0.431 | -1.18 to 0.513 |
| Sex: | | | | |
| Female | Reference | | | - |
| Male | 1.19 (0.59) | 1.99 | 0.052 | -0.13 to 2.39 |
| Body Mass (kg): | 0.13 (0.09) | 1.51 | 0.137 | -0.44 to 0.312 |

SE: Standard error; CI: Confidence Interval; *: statistically significant as P < 0.05.

Table 8. Regression coefficients and their standard errors from a linear regression model of factors influencing the angle of lumbosacral junction (LSJ°) in greyhounds. n=53.

| Explanatory Variable | Coefficient (SE) | t | P- value | 95% CI |
|----------------------|------------------|-------|----------|----------------|
| Intercept | 165.7 (8.16) | 20.3 | <0.01* | |
| Type of Sacrum: | | | | |
| Fused | Reference | | | - |
| Standard | -3.18 (1.40) | -2.27 | <0.028* | -6.0 to -0.358 |
| Sex: | | | | |
| Female | Reference | | | - |
| Male | -2.15 (1.99) | -1.08 | 0.285 | -6.15 to 1.85 |
| Body Mass (kg): | 0.07 (.29) | 0.244 | 0.808 | -0.52 to 0.66 |

SE: Standard error; CI: Confidence Interval; *: statistically significant as P < 0.05.

Table 9. Regression coefficients and their standard errors from a logistic regression model of factors influencing the length of the L.7 vertebra (presence of fused sacra) in greyhounds.

| Explanatory Variable | Fused | Total | Coefficient (SE) | z | P- value | OR (95% CI) |
|----------------------|-------|-------|------------------|-------|----------|-------------|
| Intercept | 22 | 55 | -1.2 (3.3) | 0.132 | 0.717 | |
| Sex: | | | | | | |

| | | | | | | |
|-----------------|----|----|--------------|-------|-------|----------------------|
| Female | 11 | 27 | Reference | | | - |
| Male | 10 | 26 | -0.24 (0.82) | <0.08 | 0.765 | 0.784 (0.158 to 3.9) |
| Body Mass (kg): | 55 | 53 | 0.03 (0.12) | <0.06 | 0.802 | 1.03 (0.814 to 1.3) |

SE: Standard error; OR: odds ratio; CI: confidence interval; *: statistically significant as $P < 0.05$.

FIGURES

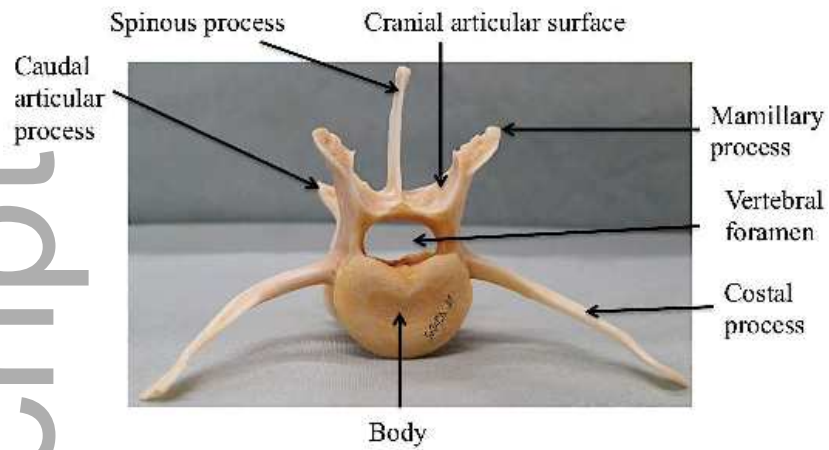


FIGURE 1 Anatomy of the 7th lumbar vertebra (L.7) in the greyhound. Author's own.

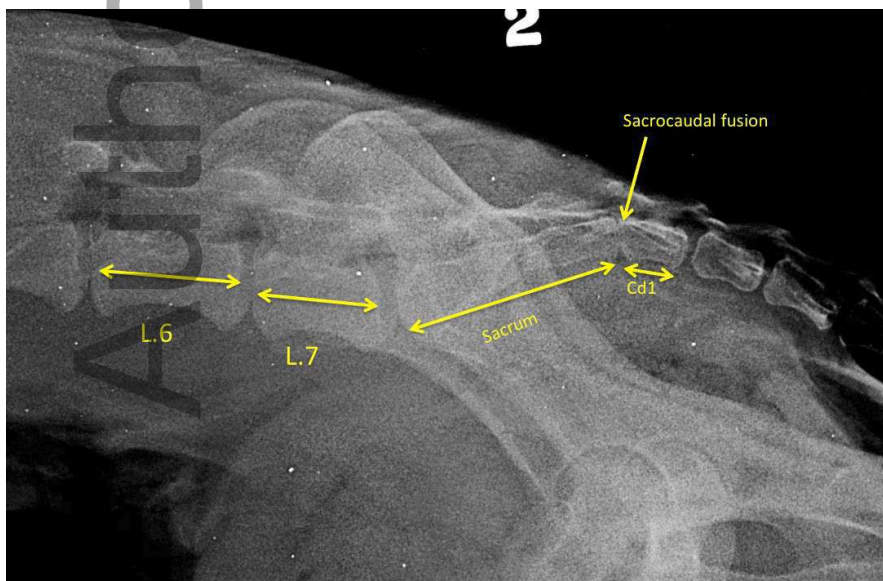
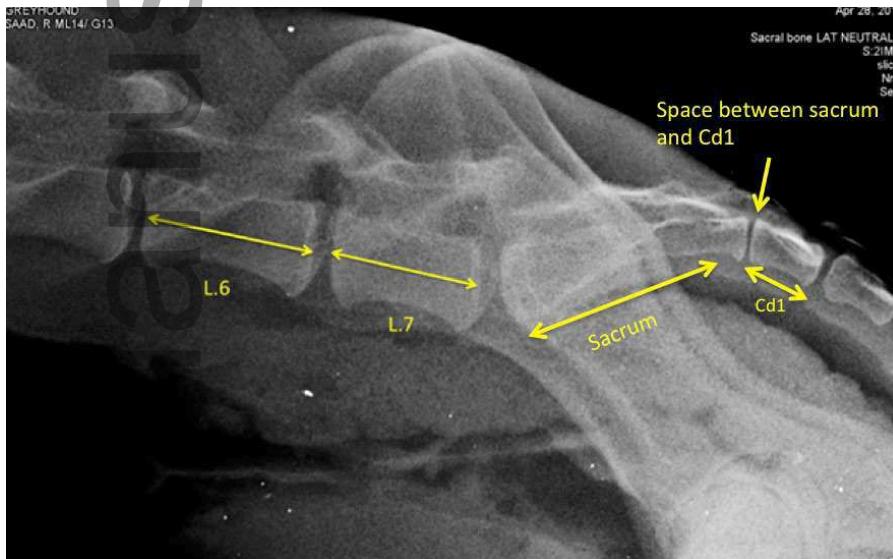


FIGURE 2 Measurements were recorded by taking the midcorpus (half way between the dorsal and ventral border of the vertebral body) length (mm) of the L.6 and L.7 vertebrae in greyhounds with standard sacrum (Top image shows joint space between S3 and Cd1 vertebrae) and those with sacrocaudal fusion (Bottom image). L.6: sixth lumbar vertebra L.7: seventh lumbar vertebra. Cd1: 1st caudal vertebra. Author's own.

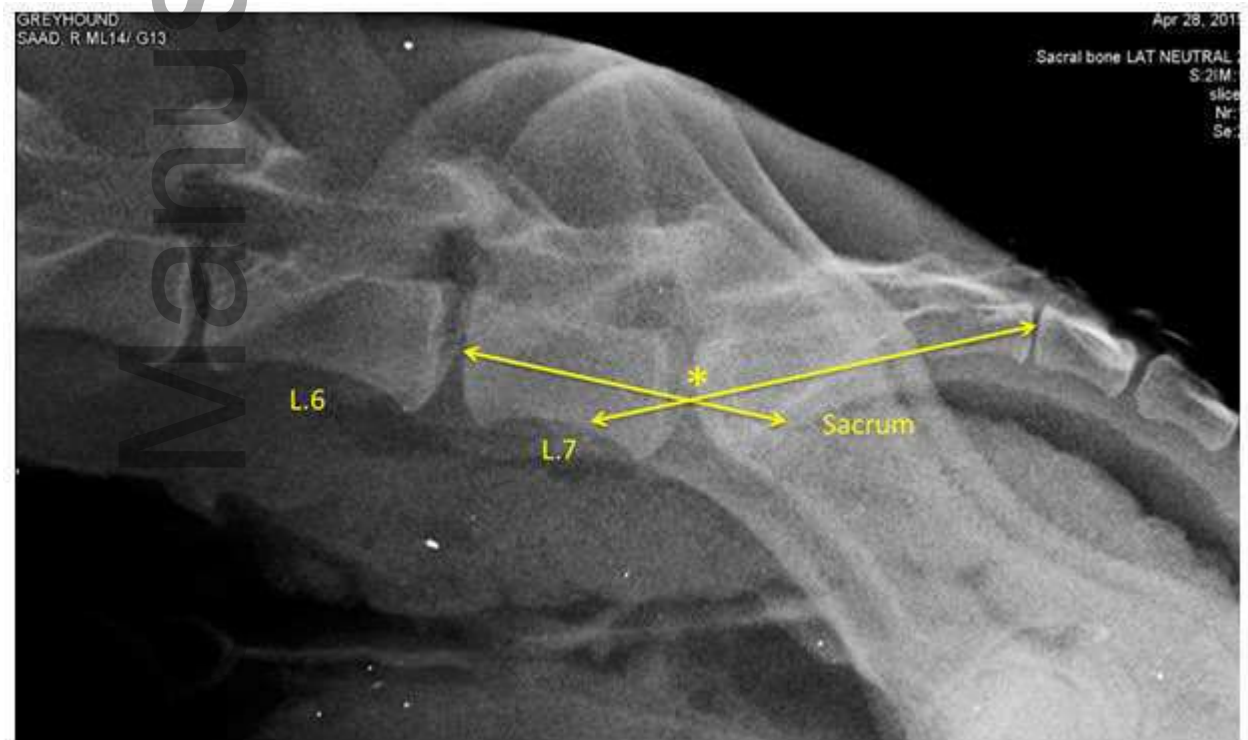


FIGURE 3 Radiograph showing how the measurements of the angle of the lumbosacral junction were taken. Angle (*) was formed by the intersection of two lines that bisect the midcorpus of the sacrum and L.7 vertebra. L.6: sixth lumbar vertebra L.7: seventh lumbar vertebra. Cd1: 1st caudal vertebra. Author's own.

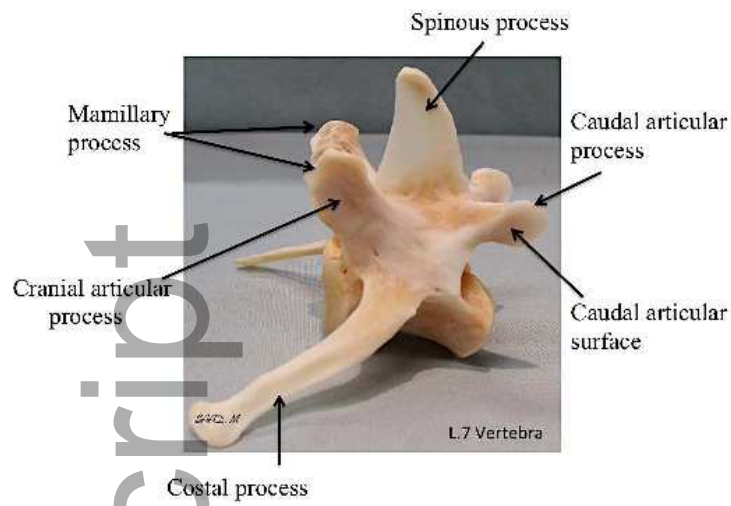


FIGURE 4 Lateral aspect of the 7th lumbar vertebra (L.7) in greyhounds with fused sacrum. Author's own.