



Canine Research

Qualitative and quantitative computed tomographic characteristics of the lumbosacral spine in German shepherd military working dogs with versus without lumbosacral pain



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ABSTRACT

Lumbosacral (LS) stenosis is a cause of lower back pain, loss of mission readiness, and early retirement in military working dogs (MWDs). Aims of the present two-part study were to evaluate a sample of German shepherd MWDs using standard clinical criteria for LS pain, standard qualitative computed tomographic (CT) criteria for LS stenosis, novel quantitative CT criteria for LS stenosis, and novel behavioral classification criteria for LS pain. Data were retrieved from archives of a tertiary referral MWD hospital. Study 1 was a retrospective, observational, two-group design with a hypothesis that there would be a significant difference in the percentage of affected German shepherd MWDs with multilevel stenosis (affecting ≥ 3 vertebrae) between LS pain groups, based on standard clinical and qualitative CT criteria. Study 2 was a retrospective, observational, cross-sectional, two- and three-group study design with a hypothesis that quantitative CT measurements would significantly differ between LS pain groups, assigned based on 3 classification systems. The 1st classification system used standard clinical criteria, while the 2nd and 3rd novel classifications included behavioral signs of LS pain. The following quantitative CT measures were recorded without knowledge of behavioral classification: vertebral foramen area, vertebral foramen volume, vertebral foramen fat area; and ratios of vertebral foramen area/vertebral body area (foramen area ratio), cranial vertebral foramen area/caudal vertebral foramen area (cranial:caudal foramen area ratio), and vertebral fat area/vertebral body area (fat area ratio). Study 1 findings did not support the hypothesis in that there was no significant difference in the percentage of dogs affected with multilevel stenosis between LS pain groups ($P = 0.6567$). Findings for study 2 supported the hypothesis in that dogs with LS pain were significantly more affected by multilevel stenosis ($P = 0.0273$). Significant differences occurred between LS pain groups in select vertebral locations for all measurements ($P \leq 0.05$) except vertebral foramen area and vertebral foramen volume ($P > 0.05$). Comparisons using novel quantitative CT measures and behavioral classification criteria identified significant differences between LS pain groups that were not detected using standard qualitative criteria. These novel quantitative and behavioral classification criteria may be helpful in future research on causes for early retirement in German shepherd MWDs.

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Introduction

Moore et al. (2001) found that one of the common causes of death or euthanasia within a population of military working dogs (MWDs) was spinal cord disease. A separate study led by Evans and colleagues (2007) found that the most common cause of discharge for a population of MWDs under the age of 5 years was behavioral problems, while spinal cord disease or behavioral problems were

the most common causes of discharge for MWDs over 5 years of age. Indrieri defined lumbosacral (LS) stenosis as a spinal disease characterized by the narrowing of the vertebral foramen and/or vertebral canal that can be aggravated by prolonged physical stress and activity in dogs. This author described the most common and consistent clinical sign of LS stenosis as inducible pain on palpation of the LS spine (Indrieri, 1988).

Multiple studies have reported the German shepherd to be one of the most common breeds to be affected by LS stenosis (Indrieri, 1988; Schulman, 1988; Chambers, 1989; Wheeler, 1992; Ness, 1994; Danielsson and Sjöström, 1999; De Risio et al., 2000, 2001; Suwankong et al., 2008; Pogorevc et al., 2016). Linn et al. (2003) reported that German shepherd dogs are often used as MWDs, and that they are bred to be stoic with a high working drive. These authors also described behavioral signs as possible early indicators of LS stenosis in MWDs; however, dogs could mask these signs (Linn et al., 2003). Jula et al. (2014) reported preliminary evidence that LS pain in MWDs could be associated with the number of affected vertebral levels and concurrent musculoskeletal lesions. Dodd et al. (2016) reported preliminary evidence that behavioral problems could be associated with multilevel LS stenosis in MWDs. Jones et al. (1996a) found evidence that experimentally induced compression of the L7 nerve root at 2 locations reduced arterial blood flow to the L7 spinal ganglion more than compression at 1 location in dogs. In addition, Mao et al. (1998) reported evidence that experimentally induced multilevel vertebral stenosis in dogs reduced the blood flow and nerve conduction velocities to affected nerve fibers. No published studies were found describing evidence that multilevel stenosis in dogs is associated with an increased severity of clinical or behavioral signs of LS pain.

Objectives for the present two-part study were to evaluate a sample of German shepherd MWDs using 1) standard clinical criteria for LS pain, 2) qualitative computed tomographic (CT) criteria for LS stenosis, 3) novel behavioral classification criteria for LS pain, and 4) quantitative CT criteria for LS stenosis. The hypothesis for the first study was that there would be a significant difference in the percentage of affected dogs across multilevel stenosis or concurrent multilevel spinal lesions groups, and between dogs with and without LS pain. Hypotheses for the second study were that CT quantitative measurements would be significantly different between LS pain groups assigned based on behavioral classification criteria, and that the presence of concurrent diseases would not affect the differences observed within the LS pain groups.

Materials and methods

The methodology of the first and second study is represented using flowcharts in Figures 1 and 2, respectively.

Study 1

Subject selection criteria

This study was a retrospective, observational, two-group study design. An ACVR-certified veterinary radiologist (JJ) searched the database of a tertiary referral MWD hospital (LTC Daniel E. Holland Military Working Dog Hospital, Lackland Joint Base, Texas; IACUC Exempt Protocol Number: 2019-04) for dogs that had CT scans of the LS region. Digital Imaging and Communication in Medicine (DICOM) CT image files and hard copies of medical records were retrieved. Medical record forms describing the following information were copied: dog duty status, descriptions of physical examination findings, descriptions of other medical and/or

behavioral problems, reasons dog was presented for LS CT scanning, results of LS radiographs and CT scans, descriptions of surgical and/or pathologic findings, and descriptions of outcome or disposition.

Dogs were included in study 1 if they were of German shepherd breed and if they were scanned during the period of September 2006 to June 2016. Dogs were excluded if medical record or CT data were unavailable, or if there was evidence of previous spinal surgery in the CT scan. Decisions for inclusion or exclusion were based on a consensus of an undergraduate student (AB), a graduate student (KBG), and an ACVR-certified veterinary radiologist (JJ) (Table 1).

Medical record data recording

An undergraduate student (AB) who was unaware of CT findings reviewed the medical record copies and recorded the following information: dog identification number, date of birth, weight, sex, date of CT scan, age when scanned, reason for CT scanning, and entries describing clinical signs of LS pain. Dogs with medical record entries explicitly stating that LS pain was elicited during LS palpation, a lordosis test, or tail manipulation were assigned to the "LS pain positive" group. Those without medical record entries describing clinical signs of LS pain were assigned to the "LS pain negative" category. Decisions for group assignments were based on a consensus between the undergraduate student and a graduate student (KBG).

Qualitative CT data recording

CT scan data were retrieved and reinterpreted by a veterinary radiologist (JJ) using imaging analysis freeware (The Horos Project; Version 2.4.0, horosproject.org) and an image analysis workstation (Mac Pro, Apple Inc., Cupertino, CA).

An undergraduate student (AB) recorded the radiologist's interpretations for number of total thoracic, lumbar, sacral, and caudal levels; as well as the number of affected vertebral locations for the following characteristics: articular process degenerative joint disease, articular process dysplasia, discospondylitis, intra-vertebral foraminal stenosis, fractures, herniated disc, neoplasia, osteochondrosis, Schmorl's nodes, spondylolisthesis, stenosis, and synovial cysts. For hip dysplasia, injury to the iliopsoas muscle, and sacroiliac degenerative joint disease characteristics, the number of available locations were recorded as 0, 1, or 2. For example, if both sacroiliac joints were included in the CT scan, then the number of available locations was recorded as "2". If a respective location for these three variables was available, then the number of affected levels was recorded as either 0, 1, or 2. For example, if both sacroiliac joints had CT lesions, then the number of affected levels was recorded as "2". Criteria for determining presence of lesions were based on previous publications (Oliver et al., 1978; Denny et al., 1982; Schulman, 1988; Watt, 1991; Lang et al., 1992; Gaschen et al., 1995; Jones et al., 1996b; Danielsson and Sjöström, 1999; Auger et al., 2000; Jones et al., 2000; Schwarz et al., 2000; Hanna, 2001; Linn et al., 2003; Michal et al., 2004; Rossi et al., 2004; Rossmesl et al., 2004; Scharf et al., 2004; Penderis et al., 2005; Forterre et al., 2006; Suwankong et al., 2006; Sale and Smith, 2007; Gödde and Steffen, 2007; Suwankong et al., 2008; Mathis et al., 2009; Archer et al., 2010; De Decker et al., 2010; Baltzer et al., 2012; Tellegen et al., 2015; Schmökel and Rapp, 2016; Mukherjee et al., 2017; Skytte and Schmökel, 2017).

For stenosis and spinal lesions other than iliopsoas injury, sacroiliac degenerative joint disease, and hip dysplasia, the number of affected levels by a given lesion was classified as "multilevel" if the lesion was present in three or more vertebral locations. If the lesion was located in one or two vertebral levels, then the number of affected levels of that dog was classified as "nonmultilevel". Figure 3 illustrates an example of a dog classified with multilevel stenosis.

Qualitative data analysis

With the assistance of a statistician (WB), the undergraduate student used spreadsheet software to calculate and graphically compare the percentage of affected versus nonaffected dogs for multilevel stenosis and multilevel lesions in each of the LS pain groups (Excel, Microsoft Corp, Cupertino, CA). The same software was also used to graphically compare hip dysplasia, injury to the iliopsoas, and sacroiliac degenerative joint disease in each of the LS pain groups. An ANOVA was used to compare percentages of affected dogs among LS pain groups based on multilevel stenosis and multilevel lesions (JMP Pro13, SAS, Cary, NC). A follow-up Student's *t*-test was used to make pairwise comparisons among the groups. *P*-values less than 0.05 were considered evidence for statistical significance.

Study 2

Subject selection criteria

This was a retrospective, observational, cross-sectional, two- and three-group study design. The medical records and CT scans of German shepherd dogs previously included in the first study were

also eligible for the second study. In addition to the dogs included in the first study, the second study also included German shepherd dogs with available LS CT scans and medical records during the period of July 2016 to January 2017 (IACUC Exempt Protocol Number: 2019-04). CT scans were selected for analyses if they were taken in the extension position, included at least one standard algorithm image set, and included images of the fourth lumbar (L4) and first sacral (S1) vertebrae. The decisions for exclusion were based on a consensus between a graduate student (KBG) and an ACVR-certified veterinary radiologist (JJ) (Table 2).

Medical record data recording

A graduate student (KBG) reviewed medical records and classified dogs into LS pain groups based on available entries recorded by military veterinarians. The method used in study 1 to classify dogs into “LS pain negative” and “LS pain positive” was repeated in study 2 and was defined as the “first pain classification”. Any “LS pain–positive” and “LS pain–negative” group assignment discrepancies between the undergraduate student for study 1 and graduate student for study 2 were resolved by discussion and consensus.

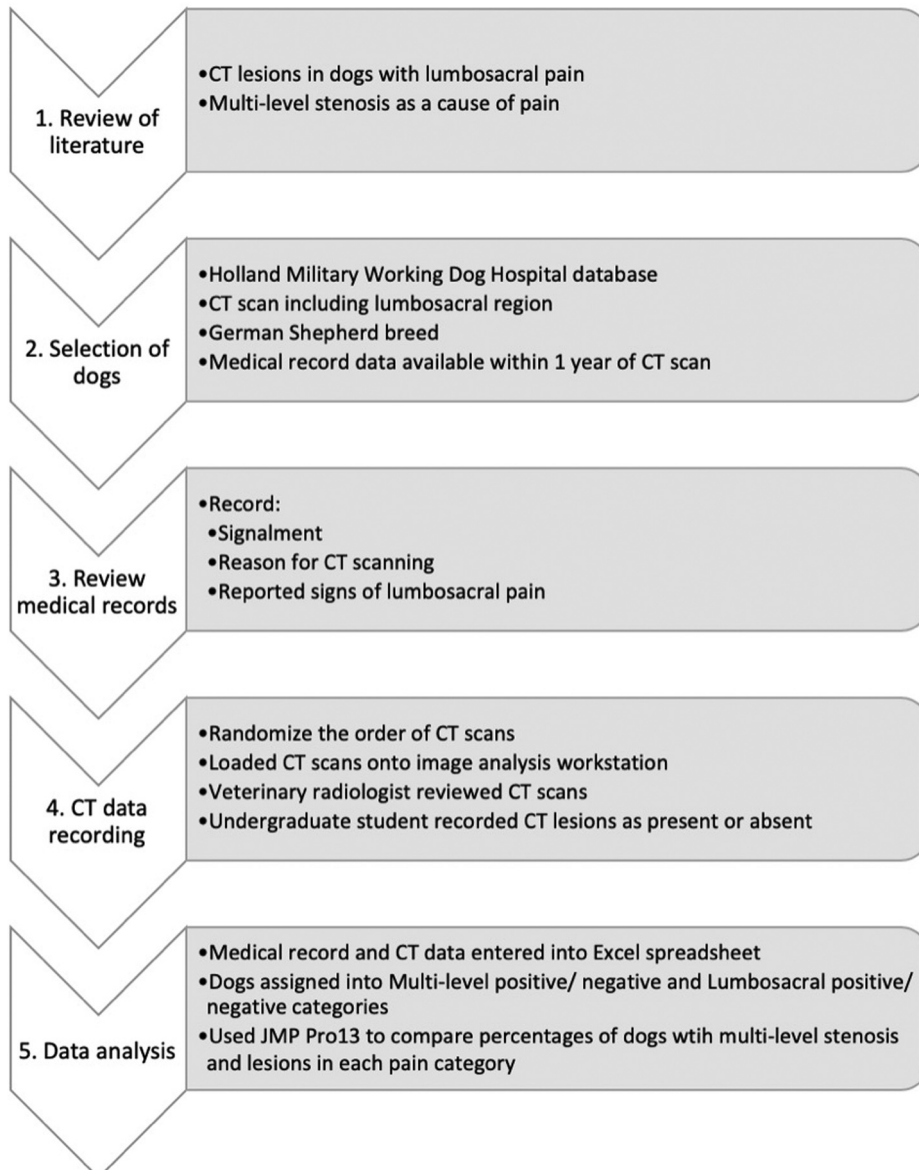


Figure 1. Study 1 flow chart of methodology.

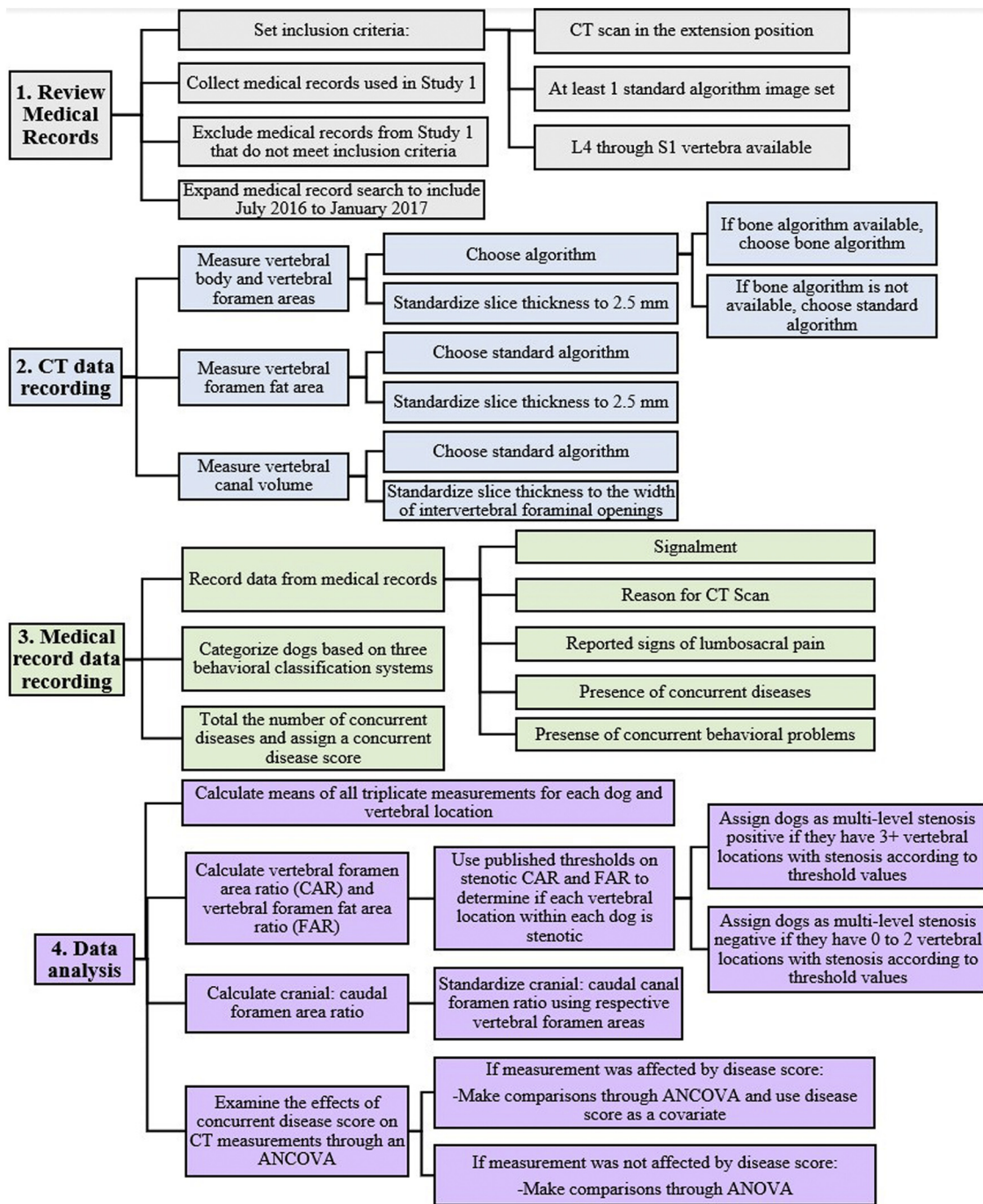


Figure 2. Study 2 flow chart of methodology.

The graduate student (KBG) also recorded the presence of all listed medical diagnoses, diseases, and behavioral problems. The onset date of these medical problems was recorded and, if present, the resolved date. Dogs that were previously placed within the “LS pain negative” category were reassigned to a “LS pain possible” group if they did not have a painful reaction to LS palpation, tail manipulation, or the lordosis test but had behavioral signs of LS pain. The following previously published signs of LS pain in dogs were recorded: reluctance to jump, use stairs, sit, perform exercise, “search high”, perform tasks, or rise; self-mutilation; expressing pain during urination or defecation; lethargy; guarding affected area; and yelping as if in pain (Tarvin and Prata, 1980; Watt, 1992;

Table 1
Reasons for dog exclusion from the qualitative analyses in the first study

Reason for exclusion	Number of dogs excluded
Continuous error occurred when loading scan	1
Discrepancies between medical record and CT scans	2
No lumbar scan available	2
No breed, age, or sex listed in medical record	1
Soft tissue mass in L7 – S1 vertebral canal with a loss of bone	1
Total	7

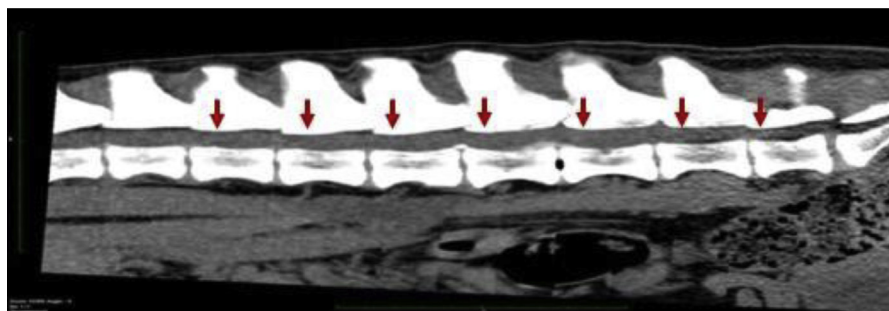


Figure 3. Mid-sagittal CT image illustrating an example of German shepherd military working dog multilevel stenosis (soft tissue window).

Ness, 1994; Gaschen et al., 1995; Danielsson and Sjöström, 1999; Hanna et al., 2000; De Riso et al., 2001; Linn et al., 2003; Scharf et al., 2004; Suwankong et al., 2006; Gööde and Steffen, 2007; Suwankong et al., 2008; Pogorevc et al., 2016). Dogs that did not have any reported clinical or behavioral signs of LS pain were retained in the “LS pain–negative” group. The dogs that were previously classified as “LS pain positive” based on their clinical signs of LS pain remained in the “LS pain positive” group. This method was defined as the “second pain classification”.

To evaluate the behavioral signs of LS pain within both LS pain groups, dogs were further categorized into one of four subgroups. Dogs without clinical or behavioral signs of LS pain were classified as “no LS pain, no behavioral signs of LS pain (NN)”. The dogs without clinical signs of LS pain but with behavioral signs of LS pain were categorized as “no LS pain, yes behavioral signs of LS pain (NY)”. Those with clinical signs of LS pain but no behavioral signs of LS pain were categorized as “yes LS pain, no behavioral signs of LS pain (YN)”. Finally, the dogs with both clinical and behavioral signs of LS pain were classified as “yes LS pain, yes behavioral signs of LS pain (YY)”. This method was defined as the “third pain classification”.

“Concurrent diseases” were defined as any medical problem or disease listed within the medical record that was present during the occurrence of behavioral and clinical signs of LS pain and/or date of CT scanning. Table 3 lists the concurrent diseases that were described in the available medical records, as well as the number of dogs in each of the four LS pain subgroups (i.e., NN, NY, YN, YY) found to have each concurrent disease. The graduate student totaled the number of concurrent diseases per dog in a spreadsheet (Excel, Microsoft Corp., Cupertino, CA). A “concurrent disease score”, ranging from 0 through 3, was assigned to each dog based on the total number of concurrent diseases. A score of “0” included dogs with 0 concurrent diseases at the time of behavioral signs, clinical signs, or date of CT scan. Dogs receiving a score of “1” had 1 to 3 concurrent diseases. Those with 4 to 6 concurrent diseases received a score of “2”, whereas dogs with more than 7 concurrent diseases were assigned a score of “3”.

Table 2

Reasons for dog exclusion from the quantitative analyses in the second study

Reason for exclusion	Number of dogs excluded
Excluded from qualitative data collection (i.e. first hypothesis/objective)	7
Continuous error occurred when loading scan	3
Complete view of L4 through S1 vertebrae not available	11
Noncontrast scans unavailable	1
Scans taken in the flexion position	1
Total	23

Quantitative computed tomography data recording

Five dogs meeting the inclusion criteria for this second study were randomly chosen for a pilot study to evaluate the effects of CT algorithm on quantitative measurements. In this pilot study, vertebral body and vertebral foramen measurements made within a bone or standard algorithms using a similar slice thickness were not found to be significantly different. For vertebral foramen volume measurements, the pilot study revealed that the values from a bone or standard algorithms were significantly different for the L6, L7, and combined total values. A more detailed description of the pilot study methods is available in the MS thesis (<https://tigerprints.clemson.edu/etd/>).

Based on the pilot study results, a slice thickness of 2.5 millimeters (mm) was used for all subsequent vertebral body, vertebral foramen, and vertebral foramen fat area measurements within the present study. In addition, vertebral foramen fat areas were measured using standard algorithm studies because the pilot study revealed that bone algorithm studies introduced variation and “noise” into the vertebral foramen fat area measurements (Figure 4). Furthermore, the pilot study revealed that vertebral canal volume measurements taken within either a standard algorithm or a bone algorithm were significantly different between algorithms, and that the endpoint of the S1 vertebral foramen was difficult to definitively determine. Therefore, the standard algorithm was chosen for the present study because it was available for all dogs and the S1 vertebral foramen was not included in the volume measurements.

The graduate student (KBG) was blinded to qualitative CT findings and medical record findings at the time of quantitative CT data recording. All quantitative measurements were measured in triplicate, with the order of scans randomized each round using a random number generator (www.random.org). A gap of one week was used between each round of measurements to minimize bias. Detailed, step-by-step instructions regarding vertebral foramen area, vertebral body area, vertebral foramen fat area, and vertebral canal volume are available in the MS thesis (<https://tigerprints.clemson.edu/etd/>).

The “pencil tool” was used to trace the outline of the vertebral body and foramen in the transverse plane, with images generated using a three-dimensional, multiplanar (3D MPR) software tool (Horos, Version 2.4.0, horosproject.org) (Figure 5). All transverse vertebral foramen areas and vertebral body areas were measured with a bone window display (window length = 300; window width = 150) using standard algorithm studies or bone algorithm studies if available. All vertebral body and foramen areas were recorded in cm².

Vertebral foramen fat area was measured using a previously described “grow region, segmentation” method, using standard algorithm studies and a soft tissue window display (window length = 40; window width = 350) (Mukherjee et al., 2017). Vertebral foramen fat area was measured from transverse CT images displayed

Table 3

Concurrent diseases present during date of CT scan and behavioral signs of pain

Concurrent diseases	NN	NY	YN	YY
Arthritis				
Coxofemoral osteoarthritis	0	0	1	1
Elbow osteoarthritis	1	0	0	0
Facet joint osteoarthritis	0	0	0	1
Hip arthritis	0	0	0	1
Sacroiliac joint arthritis	0	0	1	0
Total arthritis concurrent diseases	1	0	2	3
Allergy				
Food	0	0	2	0
Mite	0	0	0	1
Total allergy concurrent diseases	0	0	2	1
Behavior				
Situational repetitive behavior	1	0	0	0
Social distress in training	0	0	0	1
Total behavior concurrent diseases	1	0	0	1
Cardiovascular				
Azotemia	1	0	0	0
Gallop rhythm	0	0	0	1
Neutropenic leukopenia	0	1	0	0
Total cardiovascular concurrent diseases	1	1	0	1
Degenerative joint disease (DJD)				
Coxofemoral DJD	1	0	2	0
Facet joint DJD	1	0	1	2
General DJD	1	0	1	0
Hip DJD	0	0	2	0
Sacroiliac DJD	0	1	0	0
Total DJD concurrent diseases	3	1	6	2
Ear				
Otitis externa	0	1	0	0
Eye				
Cataract	0	0	1	0
Lipid deposit within cornea	0	1	0	0
Retinal dysplasia	0	0	1	0
Plasmoma	0	0	0	1
Total eye concurrent diseases	0	1	2	1
Gastro-intestinal				
Small intestine bacterial overgrowth	0	0	1	1
Tenesmus- mega colon	0	0	1	0
Total gastrointestinal concurrent diseases	0	0	2	1
Immune				
Thrombocytopenia with steroid hepatopathy	2	0	0	0
Intervertebral disc				
Intervertebral disc degeneration	0	0	1	1
Intervertebral disc disease	0	1	1	0
Intervertebral disc protrusion	2	4	5	5
Total intervertebral disc concurrent diseases	0	5	7	6
Infection				
Chagas positive	0	0	1	1
Giardia positive	1	0	0	0
Total infection concurrent diseases	1	0	1	1
Nerves				
Cauda equina compression	0	0	0	1
Lower motor neuron disease	0	0	1	0
Nerve compression/impingement	0	1	2	0
Neuritis	1	0	0	2
Total nerve concurrent diseases	1	1	3	3
Neurological				
Seizure	2	0	0	1
Oral				
Buccal masses	0	0	0	1
Bruised crowns	0	0	1	0
Total oral concurrent diseases	0	0	0	1
Reproductive system				
Enlarged prostate	1	0	2	0
Prostate cyst	0	1	0	0
Total reproductive concurrent diseases	1	1	2	0
Skin				
Alopecia	1	0	0	0
Inclusion cyst	0	0	1	0
Total skin concurrent diseases	1	0	1	0
Spinal				
Congenital malformation	0	0	0	1
Discospondylitis	0	0	1	1
Degenerative lumbosacral stenosis	0	4	7	6

(continued on next page)

Table 3 (continued)

Concurrent diseases	NN	NY	YN	YY
Dural mineralization	1	2	2	1
Ligamentous hypertrophy	0	1	0	0
Neuroforaminal stenosis	1	0	2	3
New bone formation on vertebrae	0	0	0	2
Osteochondrosis	1	0	0	0
Retrolisthesis	0	1	0	0
Spondylosis	2	2	3	5
Schmorl's node	0	1	0	0
Synovial cyst	0	0	1	1
Telescoping of articular facets	0	0	0	1
Transitional vertebrae	0	1	0	0
Unfused sacrum	0	0	0	1
Total spinal concurrent diseases	5	12	16	21
Tail issues				
Caudectomy/tail tip amputation	1	2	2	2
Recent history of tail tip trauma	1	2	1	2
Total tail-related concurrent diseases	2	4	3	4
Other musculoskeletal concurrent diseases				
Concurrent heat injury	0	1	1	0
Cranial cruciate ligament rupture	1	0	0	0
Elbow dysplasia	0	1	0	0
Interdigital funiculitis	1	0	0	0
Inguinal hernia	1	0	0	0
Thin body conformation	0	1	0	0
Total "other" concurrent diseases	3	3	1	0

NN, no LS pain, no behavioral signs of LS pain; NY, no LS pain, yes behavioral signs of LS pain; YN, yes LS pain, no behavioral signs of LS pain; YY, yes LS pain, yes behavioral signs of LS pain.

"Concurrent diseases" were defined as any medical problem or disease listed within the medical record that was present during the occurrence of behavioral and clinical signs of lumbosacral (LS) pain and/or date of CT scanning.

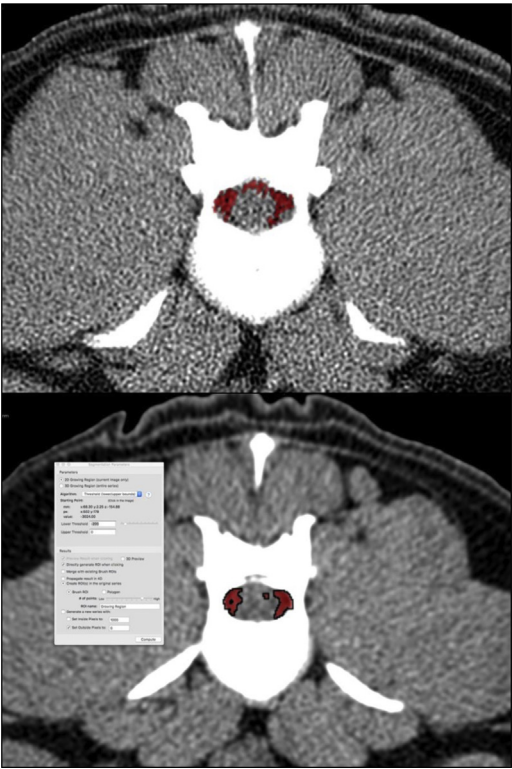


Figure 4. Top: Transverse CT image illustrating the “grow region” segmentation method for calculating vertebral foramen fat area measurements and severe image noise caused by use of a bone algorithm. (2.5 mm slice thickness, 350 window length: 40; window width). Bottom: Transverse CT image illustrating a vertebral fat area measurement using a “grow region” segmentation tool, 2.5 mm slice thickness and a standard algorithm (350 window length: 40; window width). Notice the reduction in noise using this algorithm.

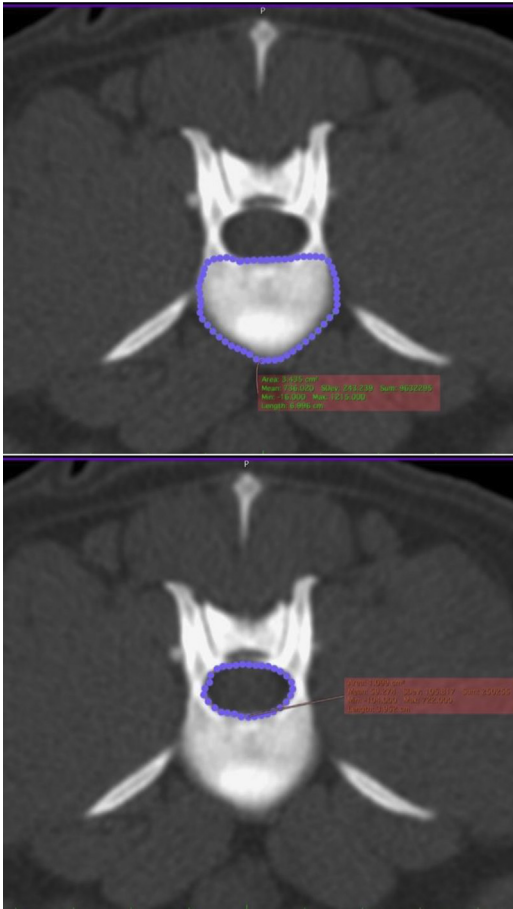


Figure 5. Top: Transverse CT image illustrating a vertebral body area measurement using 2.5 mm slice thickness and a standard algorithm (1500 window width, 300 window level). Bottom: Transverse CT image illustrating a vertebral foramen area measurement using 2.5 mm slice thickness and a standard algorithm (1500 window width, 300 window level).

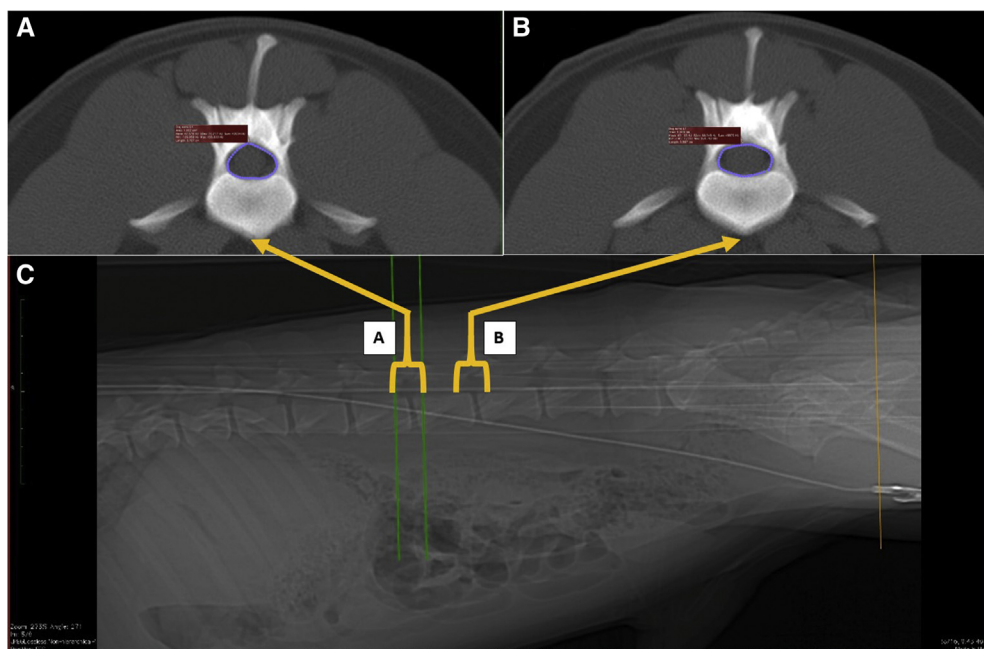


Figure 6. Computed tomography images illustrating techniques used for tracing regions of interest (ROIs) for calculating the L4 vertebral foramen volume (bone window, standard algorithm). A: Thick slab (15 mm) transverse image illustrating the ROI tracing for calculation of the cranial L4 vertebral foramen volume. B: Thick slab (15 mm) transverse image illustrating the ROI tracing for calculation of the caudal L4 vertebral foramen volume. C: Sagittal CT localizer image illustrating the start and end points that were used for determining the “thick slab” slice thickness settings for A and B above. To calculate the vertebral canal volume, regions of interest (ROIs) were first created in each transverse slice by tracing the boundaries of each vertebral foramen. At the cranial and caudal portions of the vertebral foramen, the “thick slab” tool was used to adjust the slice thickness until the intervertebral foramina were eliminated (Figure 6A, 6B). To determine this slice thickness, the starting and ending points were set at the cranial and caudal borders of the intervertebral foramina (Figure 6C). After all these ROIs were created, the ROI volume tool was used to sum them, generate a 3D image of the vertebral canal (Figure 7), and calculate a 3D volume for the vertebral canal.

using the 2D software tool because the 3D MPR software tool did not allow the use of segmentation. The vertebral location was determined by referencing a localizer or sagittal image of the spine on the adjacent screen and using the software’s reference bar as a location identification. The “grow region” segmentation tool was used to isolate and measure the area of epidural fat with an upper and lower threshold bound of 0 Hounsfield units (HU) and -200 HU, respectively. The cursor was used to select all portions of the vertebral foramen and identify all the possible locations with epidural fat (Figure 4). Only tissues that fell within the designated threshold were selected by the program, and any vertebral foramen fat area values that were not connected were manually added together to obtain a total vertebral foramen fat area in cm^2 .

For calculations of LS region vertebral canal volume, the slice thickness for the 2D transverse CT study was first set to the maximum thickness of the intervertebral foramina of each individual dog using a localizer image display and the “thick slab” tool in the image analysis software (Figure 6). For dogs that did not have

a localizer image, a standardized slice thickness of 15 mm was used. The “pencil” tool was then used to trace the outline of the vertebral foramen in each individual transverse CT slice and create a regions of interest (ROI) for each slice. The “ROI volume tool” was used to sum all of the transverse slice ROIs, create a 3-dimensional image of the vertebral canal, and calculate the total LS region vertebral canal volume (Figure 7). All LS region vertebral canal volumes were recorded in cm^3 .

Quantitative data analysis

Data were analyzed by a graduate student (KGB) and a statistician (WB), using spreadsheet (Excel, Microsoft Corp., Cupertino, CA) and statistical analysis (JMP Pro13) software. *P*-values less than 0.05 were considered evidence for statistical significance.

The averages of all triplicate measurements for each dog were calculated using spreadsheet software (Excel, Microsoft Corp., Cupertino, CA) and used for subsequent analyses. Dogs were first compared by LS pain positive versus LS pain–negative groups using

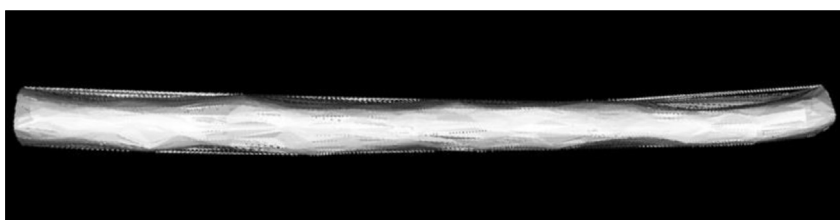


Figure 7. Reformatted 3D image of the L4-L7 vertebral canal that was used to calculate the vertebral canal volume for a dog categorized as “Yes Pain, Yes Behavioral Signs of Lumbosacral Pain” (lateral view, patient’s head to the left and tail to the right). The region of interest (ROI) volume tool in the image analysis software was used to sum the volumes that were calculated for each vertebral canal using the techniques illustrated in Figure 6.

Table 4

Sample population included in the qualitative analyses in the first study

Variable	All	LS pain positive	LS pain negative
Number of dogs	54	29	25
Average age (months)	70.87 ± 26.74 (Range: 15–116)	77.55 ± 22.69 (Range: 16–116)	63 ± 29.36 (Range: 15–114)
Sex	44 Males 10 Females	24 Males 5 Females	20 Males 5 Females
Average weight, kilograms (kg)	33.15 ± 4.40 (Range: 25–44.09)	33.86 ± 3.62 (Range: 25.91–40)	32.34 ± 5.11 (Range: 25–44.09)

LS, lumbosacral.

the first pain classification method. The vertebral foramen area ratio (CAR) was calculated by dividing the mean vertebral foramen area by the mean vertebral body area for each vertebral location (Jones et al., 1995; Mukherjee et al., 2017). To calculate vertebral foramen fat area ratio (FAR), the mean vertebral foramen fat area was divided by the mean vertebral body area for each vertebral location (Mukherjee et al., 2017).

Previously published CAR LS stenosis thresholds and FAR LS stenosis thresholds were used to determine each dog's LS stenosis status at each vertebral location (Jones et al., 1995; Mukherjee et al., 2017). These CAR and FAR thresholds were used to separately classify the vertebral location of each dog as LS stenosis positive or negative. Dogs with three or more LS stenosis positive vertebral locations, as determined by CAR thresholds and/or FAR thresholds,

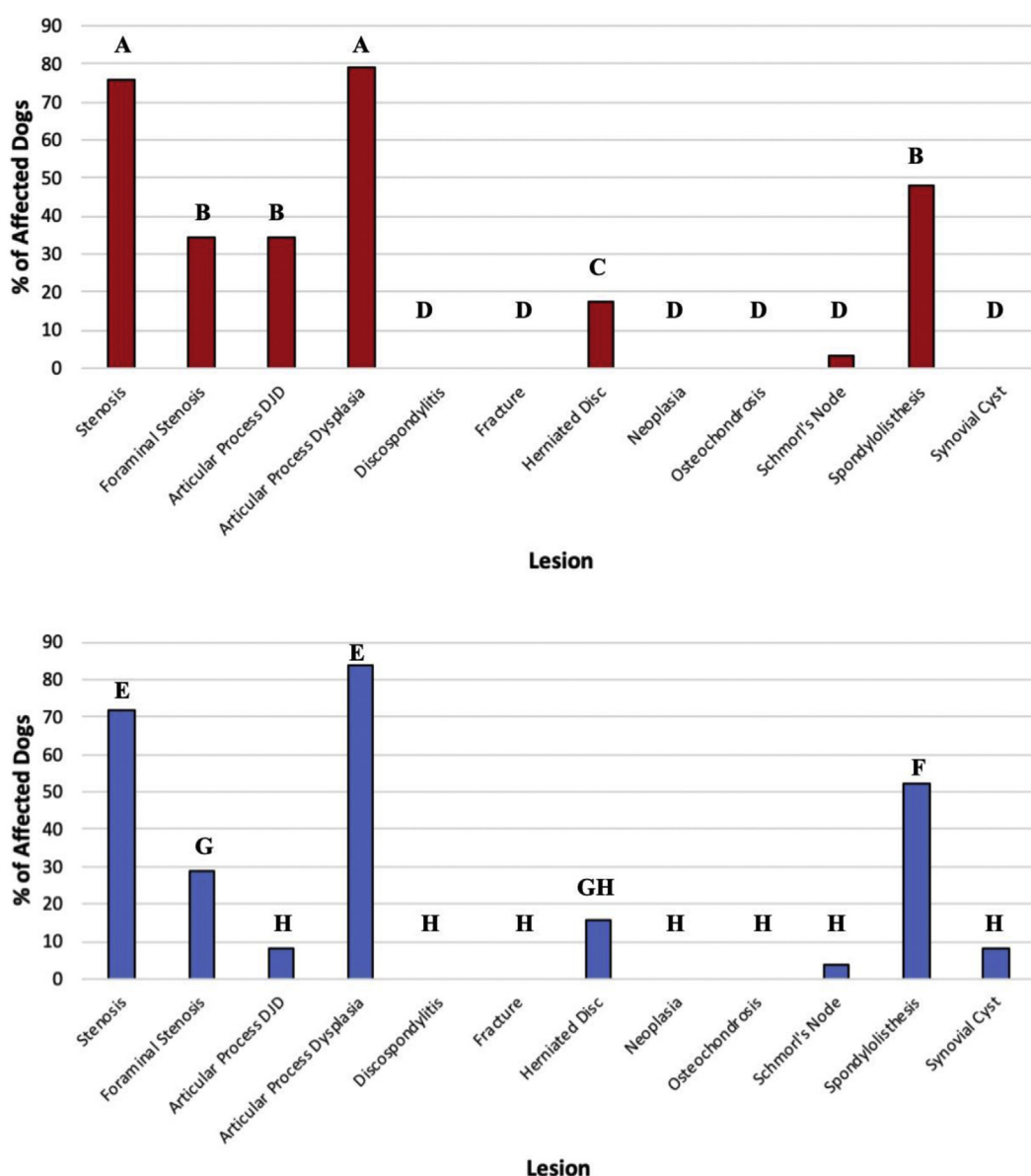


Figure 8. Graphical comparison of percentage of affected dogs with lumbosacral pain (n = 29) and without lumbosacral pain (n = 25) for each multilevel lesion. The top figure represents the percentage of affected dogs with lumbosacral pain (n = 29). The bottom figure represents the percentage of affected dogs without lumbosacral pain (n = 25). Lesions with different letters denotes a statistically significant difference using a Student's t-test comparison ($P < 0.05$).

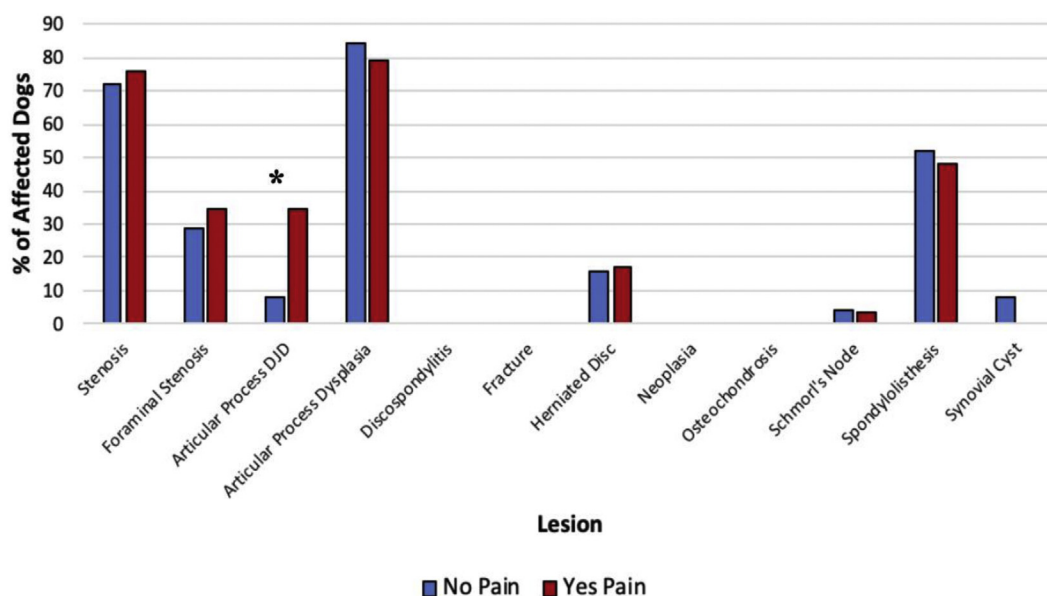


Figure 9. Graphical comparison of percentage of affected dogs with multilevel lesions between pain groups. An asterisk indicates a significant difference between pain groups through a Student's t-test comparison ($P < 0.05$).

were characterized as “(CAR) multilevel stenosis positive” and/or “(FAR) multilevel stenosis positive”, respectively. A Fisher's exact test was used to compare the number of dogs classified as multilevel stenosis through CAR or FAR stenosis thresholds to pain classification systems.

To quantify vertebral foramen “funneling”, cranial:caudal vertebral foramen area ratios were calculated by dividing the cranial vertebral foramen area ratio by the caudal vertebral foramen area ratio for all vertebral locations. Owing to the differences by weight class the cranial:caudal vertebral foramen area ratio was standardized using vertebral body area.

A Student's t-test or Wilcoxon's test were used to compare age, sex, weight, quantitative measurements, and ratios between LS pain groups; with test selection based on whether data were normally distributed.

Dogs were then grouped into three pain categories (LS pain negative, LS pain possible, and LS pain positive) using the 2nd classification method and into four pain categories (“NN”, “NY”, “YN”, and “YY”) using the 3rd classification method. An ANOVA or Kruskal-Wallis test, depending on data normality, was used to compare age, sex, weight, quantitative measurements, and ratios

between these LS pain groups. Vertebral canal volume was further analyzed with an ANCOVA with the weight in kilograms as the covariate due to the differences in weights between LS pain groups.

Finally, an additional analysis was completed to examine the effect of concurrent diseases on the results observed between groups in the three pain classifications. An ANCOVA was used with weight in kilograms, concurrent disease score, and the respective pain classification systems as covariates. A follow-up Student's t-test was used to make pairwise comparisons. These ANCOVA analyses were used during cases that the concurrent disease score significantly affected a quantitative measurement; however, the previously mentioned analyses were used in groups where the concurrent disease score had no significant effects on the results.

Results

Study 1

A total of 61 dogs met initial inclusion criteria. Seven dogs were excluded, and reasons for these exclusions are outlined in Table 1. For the final sample of 54 dogs, 29 were classified as LS pain positive

Table 5
Comparison of multilevel and nonmultilevel stenosis and lesions within pain categories

Lesion	LS pain positive		P-value	LS pain negative		P-value
	Multilevel	Nonmultilevel		Multilevel	Nonmultilevel	
Stenosis	75.86%	24.14%	<0.001	72%	28%	0.0014
Foraminal stenosis	34.48%	58.62%	0.0673	28%	44%	0.2473
Articular process DJD	34.48%	37.93%	0.7892	8%	28%	0.0679
Articular process dysplasia	79.30%	20.69%	<0.001	84%	16%	<0.001
Discospondylitis	0%	0%	N/A	0%	0%	N/A
Fracture	0%	3.45%	0.3216	0%	0%	N/A
Herniated Disc	17.24%	75.86%	<0.001	16%	56%	0.0026
Neoplasia	0%	0%	N/A	0%	0%	N/A
Osteochondrosis	0	6.90%	0.1554	0	0%	N/A
Schmorl's node	3.44%	17.24%	0.0874	4%	2%	0.0848
Spondylolisthesis	48.28%	41.38%	0.6050	52%	44%	0.5805
Synovial cyst	0	24.14%	0.0042	8%	24%	0.1279

LS, lumbosacral; DJD, degenerative joint disease.

Comparisons within pain groups completed through Student's t-test. A P-value less than 0.05 was considered to be significant. Significant P values are represented in bold.

Table 6

Comparison of affected versus nonaffected dogs for hip dysplasia, iliopsoas injury, and sacroiliac degenerative joint disease by pain category

Lesion	Lumbosacral pain positive (n = 29)		Lumbosacral pain negative (n = 25)	
	Affected	Not affected	Affected	Not affected
Hip dysplasia	4/17 23.53%	13/17 76.47	1/13 7.14%	12/13 92.31%
Iliopsoas injury	4/12 33.33%	8/12 66.67%	5/11 45.55%	6/11 54.55%
Sacroiliac degenerative joint disease	27/28 96.43%	1/28 3.57%	24/25 96%	1/25 4%

and 25 were categorized as LS pain negative (Table 4). There were 44 males and 10 females. The ages ranged from 15 to 116 months (median 70 months $\pm$ 26.74 months). Weights ranged from 25 to 44.09 kilograms (kg) (median: 33.18 kg $\pm$ 4.40 kg).

Graphical comparisons of multilevel stenosis and multilevel lesions within dogs with and without LS pain are provided in Figure 8. The percentage of affected dogs with multilevel stenosis or multilevel articular process dysplasia was not significantly different within both the LS pain–positive and LS pain–negative groups. Multilevel stenosis and multilevel articular process dysplasia affected a significantly larger percentage of dogs in both LS pain groups than the other multilevel lesions. The following multilevel lesions were not found in either LS pain group: discospondylitis; fracture; osteochondrosis.

Multilevel stenosis affected 76% of the dogs with LS pain and 72% of those without LS pain; however, the percentage of affected dogs did not differ significantly ($P = 0.6567$) (Figure 9). Articular process degenerative joint disease affected a significantly greater percentage of dogs with LS pain when compared with those without LS pain ($P = 0.0024$).

Dogs within both LS pain groups were significantly more affected by multilevel stenosis when compared with nonmultilevel stenosis ($P < 0.05$) (Table 5). In addition, both LS pain groups were significantly more affected by multilevel articular process dysplasia when compared with a nonmultilevel articular process dysplasia ($P < 0.001$). Both LS pain groups were significantly more affected by a nonmultilevel herniated disc compared with a multilevel herniated disc ($P < 0.05$). Dogs with LS pain were significantly more affected by a nonmultilevel synovial cyst when compared with multilevel synovial cyst ($P = 0.0042$). Sacroiliac degenerative joint disease affected 96.43% of dogs with LS pain and 96% of those without LS pain (Table 6).

Study 2

A total of 68 dogs met initial inclusion criteria, and 23 were excluded because of reasons outlined in Table 2. For the final

sample of 45 dogs, there were 38 males and 7 females. Ages ranged from 15 months to 116 months (median: 69 months $\pm$ 28.23 months). Weights ranged from 25 kilograms to 44.09 kilograms (kg) (median: 32.73 kg $\pm$ 4.51 kg). The signalment for the groups of dogs within the first, second, and third classification system are further detailed in Tables 7–9, respectively. The clinical and behavioral signs of LS pain reported for the groups of dogs are detailed in the Supplementary Material. There were 24 dogs classified as LS pain positive and 21 categorized as LS pain negative based on the first classification system. When using the second pain classification system, there were 24 LS pain–positive, 13 LS pain–possible, and 8 LS pain–negative dogs. For the third pain classification system, there were 8 “NN” dogs, 13 “NY” dogs, 12 “YN” dogs, and 12 “YY” dogs. For all three classification systems, groups were significantly different in age but not by weight or sex. The LS pain–positive group was significantly older than the LS pain–negative group in the first classification system. For the second system, the LS pain–positive group was significantly older than the LS pain–negative group, but not the LS pain possible group. In the third classification system, the “YN” group was significantly older than the other three groups. All LS pain groups within all three classification systems were male-dominated. The numbers of dogs receiving each concurrent disease score (i.e. 0–3) by pain classification system are detailed in Table 10.

Vertebral foramen area was significantly different between groups by weight class for all locations, as well as by sex for only the caudal L4 location. No significant differences were observed for age. When these vertebral foramen areas were converted into vertebral foramen area ratios, there were no significant differences between age, sex, or weight. Average vertebral foramen area did not differ significantly between LS pain groups in either of the three pain classification systems. Vertebral foramen area ratio was significantly affected by concurrent disease score; therefore, the analyses accounting for concurrent disease score were used for this measurement. Significant differences were only observed for cranial L5, caudal L5, cranial L6, and caudal L6 for the third pain classification for vertebral foramen area ratio (Figure 10). Finally, the stenosis threshold values obtained through vertebral foramen area ratios were not significantly different by LS pain groups.

Cranial:caudal vertebral foramen area ratio was significantly different between sex for the L4 vertebrae and was significantly different between weight groups for the L6 vertebrae. No differences were observed by age groups. Owing to the differences by weight class the cranial:caudal vertebral foramen area ratio was standardized using body area. The standardized cranial:caudal vertebral foramen area ratios were significantly affected by concurrent disease score. When comparing each ratio by vertebral location, both ratios were significantly lower in the L7 vertebrae, followed by the L6 vertebral location (Table 11). The ratios for the L4 and L5 vertebral locations were not significantly different from another and were both larger than the L6 and L7 vertebral locations.

Table 7

Sample population included in the quantitative analyses in the second study: first pain classification

First pain classification (present versus absent)				P-value
Variable	All	LS pain present	LS pain absent	
Number of dogs	45	24	21	-
Average age (months)	61.48 $\pm$ 28.23 (Range: 15–116)	70.98 $\pm$ 26.84 (Range: 16–116)	50.62 $\pm$ 26.32 (Range: 15–102)	0.0139
Sex	38 Males 7 Females	19 Males 5 Females	19 Males 2 Females	0.2064
Average weight, kilograms (kg)	33.04 $\pm$ 4.51 (Range: 25–44.09)	33.72 $\pm$ 3.73 (Range: 25.91–40.91)	32.29 $\pm$ 5.24 (Range: 25–44.09)	0.2064

LS, lumbosacral.

A P value less than 0.05 was considered to be significant.

Significant P values are represented in bold.

Table 8

Sample population included in the quantitative analyses in the second study: second pain classification

Second pain classification (present, possible, absent)					P-value
Variable	All	LS pain present	LS pain possible	LS pain absent	
Number of dogs	45	24	13	8	–
Average age (months)	61.48 ± 28.23 (Range: 15–116)	70.98 ± 26.84 ^A (Range: 16–116)	53.08 ± 25.21 ^{AB} (Range: 22–86)	46.63 ± 29.34 ^B (Range: 15–102)	0.0429
Sex	38 Males 7 Females	19 Males 5 Females	12 Males 1 Female	7 Males 1 Female	0.5332
Average weight, kilograms (kg)	33.04 ± 4.51 (Range: 25–44.09)	33.72 ± 3.73 (Range: 25.91–40.91)	33.81 ± 5.21 (Range: 25–44.09)	29.81 ± 4.53 (Range: 25–36.36)	0.0611

LS lumbosacral.

Significant differences are denoted by different letters.

A *P* value less than 0.05 was considered to be significant.Significant *P* values are represented in bold.

The standardized cranial:caudal vertebral foramen area ratio was significantly higher in the LS pain–positive dogs when compared with the LS pain–negative dogs at the L5 vertebral location for the first pain classification. Within the second pain classification, the standardized cranial:caudal vertebral foramen area ratios of the LS pain–negative and LS pain–possible groups were significantly smaller than the ratios of the LS pain–positive group at the L5 vertebral location. For the L5 vertebral location within the third pain classification, the standardized cranial:caudal vertebral foramen area ratios of the “NN” and “NY” LS pain groups were not significantly different from each other and the ratios were significantly smaller than the “YY” group. The ratios of the “YN” group was not significantly different from either of the “NN”, “NY”, or “YY” LS pain groups. There were no other ratio differences observed for any other vertebral locations within the third pain classification.

Vertebral foramen fat area was significantly different between sexes at the caudal L7 location and was significantly different between weight classes at the following locations: caudal L4, cranial L5, caudal L5, cranial L6, caudal L6, cranial L7, and caudal L7. No differences were observed between age groups. When vertebral foramen fat area was converted into a vertebral foramen fat area ratio, there were no significant differences between sex, weight, or age. Vertebral foramen fat area was significantly different between LS pain–positive and LS pain–negative dogs at the cranial L5, cranial L7, and cranial S1 locations; however, no significant differences were observed within the vertebral foramen fat area ratios. Interestingly, the vertebral foramen fat area within the cranial L5 was significantly higher in the LS pain–positive group versus the LS pain–negative group within the first pain classification, but the opposite was true for the caudal L7 and cranial S1 locations. Significant differences did occur between LS pain groups of the second pain classification system for vertebral foramen fat area at cranial

L5 and cranial S1 locations, as well as for vertebral foramen fat area ratio at the caudal L7 and cranial S1 locations (Figure 11). Furthermore, significant differences were observed between LS pain groups at cranial L5, caudal L7, and cranial S1 for vertebral foramen fat area within the third classification, as well as at caudal L6, caudal L7, and cranial S1 for vertebral foramen fat area ratio (Figure 12). Finally, the stenosis thresholds determined through vertebral foramen fat area ratios were significantly different between groups for only the first pain classification system, where the LS pain–positive group had more dogs affected by multilevel stenosis than the LS pain–negative group (Figure 13). Comparisons within the other two LS pain classifications were not possible because of a low sample size within LS pain groups.

Volume was significantly different between sexes for L4, L5, and “all” locations. In addition, volume measurements were significantly different between weight classes for all vertebral locations; however, age groups were not significantly different from each other. Volume did not differ significantly between LS pain or behavior groups. Because there was a difference between weight classes, additional analyses were conducted that analyzed LS pain groups while controlling for weight; however, no differences were found.

Discussion

Study 1

An unexpected finding within study 1 was that the percentage of dogs with multilevel stenosis was not significantly different between LS pain groups; however, dogs within both LS pain categories were significantly more affected by multilevel stenosis when compared with nonmultilevel stenosis. It is possible that some German shepherd MWDs had pre-existing multilevel stenosis before the onset of detectable signs of LS pain, or that some of the

Table 9

Sample population included in the quantitative analyses in the second study: third pain classification

Third pain classification (NN, NY, YN, YY)						P-value
Variable	All	NN	NY	YN	YY	
Number of dogs	45	8	13	12	12	–
Average age (months)	61.48 ± 28.23 (Range: 15–116)	46.63 ± 29.34 ^B (Range: 15–102)	53.08 ± 25.21 ^B (Range: 22–86)	84.84 ± 23.64 ^A (Range: 47.04–116.04)	57.79 ± 23.76 ^B (Range: 15.96–87)	0.0054
Sex	38 Males 7 Females	7 Males 1 Female	12 Males 1 Female	8 Males 4 Females	11 Males 1 Female	0.2580
Average weight, kilograms (kg)	33.04 ± 4.51 (Range: 25–44.09)	29.81 ± 4.53 (Range: 25–36.36)	33.81 ± 5.21 (Range: 25–44.09)	33.74 ± 3.71 (Range: 25.91–39.09)	33.70 ± 3.91 (Range: 6.82–40.91)	0.6222

“NN”, lumbosacral pain negative without behavioral signs of pain; “NY”, lumbosacral pain negative with behavioral signs of pain; “YN”, lumbosacral pain positive without behavioral signs of pain; “YY”, lumbosacral pain positive plus behavioral signs of pain.

A *P* value less than 0.05 was considered to be significant.Significant *P* values are represented in bold.

Table 10
Number of dogs in each concurrent disease score (0–3) by pain classification

Pain classification	0	1	2	3
Classification 1				
Lumbosacral pain negative (n = 21)	3	12	5	1
Lumbosacral pain positive (n = 24)	0	10	11	3
Classification 2				
Lumbosacral pain negative (n = 8)	1	4	2	1
Lumbosacral pain possible (n = 13)	2	8	3	0
Lumbosacral pain positive (n = 24)	0	10	11	3
Classification 3				
NN (n = 8)	1	4	2	1
NY (n = 13)	2	8	3	0
YN (n = 12)	0	5	5	2
YY (n = 12)	0	5	6	1

“NN”, lumbosacral pain negative without behavioral signs of pain; “NY”, lumbosacral pain negative with behavioral signs of pain; “YN”, lumbosacral pain positive without behavioral signs of pain; “YY”, lumbosacral pain positive plus behavioral signs of pain.

A “concurrent disease score”, ranging from 0 through 3, was assigned to each dog based on the total number of concurrent diseases. A score of “0” included dogs with 0 concurrent diseases at the time of behavioral signs, clinical signs, or date of CT scan. Dogs receiving a score of “1” had 1 to 3 concurrent diseases. Those with 4 to 6 concurrent diseases received a score of “2”, while dogs with more than 7 concurrent diseases were assigned a score of “3”.

“LS pain–negative” dogs were experiencing LS pain, but it was not detected within clinical examinations. Jones et al. (1995) proposed that the presence of developmental or congenital multilevel stenosis could be a predisposing factor for LS pain and other clinical signs in dogs with later onset acquired or degenerative LS stenosis. Findings from the present study also indicated that the multilevel stenosis phenotypic trait was present with a high frequency in this sample of German shepherd MWDs.

Degenerative disease of articular process joints has been considered a cause of LS pain in humans for decades (Lewinnek and Warfield, 1986). The results of the present study suggest that dogs with or without LS pain could have multiple concurrent spinal lesions; however, articular process degenerative joint disease was more commonly found in dogs with LS pain.

Within a study conducted by Künzel et al. (2002), German shepherd dogs were the most common breed affected by degenerative sacroiliac joint disease, such as osteophyte formation and other lesions within the extra-synovial space of the joint. This

previous finding supports the present study's results, suggesting that degenerative sacroiliac joint disease may be present in German shepherd dogs with or without LS pain.

A limitation of study 1 was the inability to control how many vertebral locations were scanned in each dog. It was therefore not possible to compare groups based on continuous data such as percentages of affected vertebral locations. Instead, lesions between and within LS pain groups were compared based on categorical data such as the percentages of dogs with versus without multilevel stenosis (defined as ≥ 3 locations). A second limitation of study 1 was the use of medical record excerpts rather than complete medical records. The effects of this limitation were minimized as much as possible by using standardized phrases and words to select medical record entries and categorize dogs into LS pain categories. This method of medical record review reflects the real-life situation that some MWD veterinarians face when providing consultations. As with any retrospective study, another limitation was the choice to assign dogs as “LS pain negative” if there was a lack of medical record entries describing signs of LS pain. The possibility cannot be completely excluded that some dogs with LS pain could have been present in that group. Entries describing signs of LS pain could have been lacking in some dogs because clinicians were focused on other clinical problems and were not specifically checking dogs for LS pain.

Study 2

Studies conducted by Jones et al. (1995) and Mukherjee et al. (2017) found that the transverse vertebral foramen areas were not significantly different between LS stenosis positive and LS stenosis–negative groups of dogs. Results within the present study supported these previous findings in that average vertebral foramen areas between LS pain groups of either of the three pain classification systems did not differ significantly from each other. However, when these vertebral foramen areas were converted into vertebral foramen area ratios, Mukherjee et al. (2017) did not find any differences between LS stenosis groups, whereas the study conducted by Jones and colleagues found that these ratios were significantly smaller in dogs with LS stenosis for two vertebral locations. A possible explanation for the discrepancies between the previous two studies and the current study could have been the sample populations of dogs. Jones et al. (1995) sampled dogs of

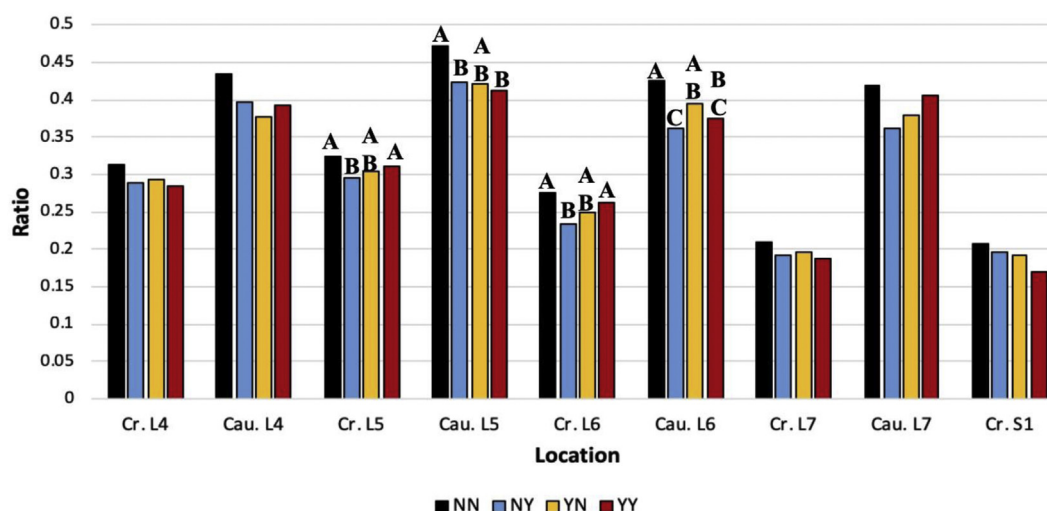


Figure 10. Graphical comparison of the vertebral foramen area ratio: Third pain classification. Significant differences determined through an ANOVA and pairwise Student's t-tests are denoted by a difference in lettering ($P < 0.05$). “NN”, lumbosacral pain negative without behavioral signs of pain; “NY”, lumbosacral pain negative with behavioral signs of pain; “YN”, lumbosacral pain positive without behavioral signs of pain; “YY”, lumbosacral pain positive plus behavioral signs of pain. “Cr.”, cranial; “Cau.”, caudal.

Table 11

Location differences for cranial:caudal vertebral foramen area ratio and standardized cranial:caudal vertebral foramen area ratio

Variable	L4	L5	L6	L7	P-value
Cranial:caudal vertebral foramen area ratio	0.8871 ^A	0.8792 ^A	0.8040 ^B	0.6716 ^C	<0.001*
Standardized cranial:caudal vertebral foramen area ratio	0.7393 ^A	0.7205 ^A	0.6606 ^B	0.4966 ^C	<0.001*

Significant differences determined through pairwise Student's t-test are denoted by different letters ($P < 0.05$).

Significant P values are represented in bold.

varying breeds and ages that were not MWDs, whereas Mukherjee et al. (2017) sampled Labrador Retriever MWDs that were all less than 5 years of age. The previous two studies also classified their dogs as LS pain positive and LS pain negative based only on standard clinical criteria, whereas the present study categorized dogs using both clinical and behavioral criteria for LS pain (Jones et al., 1995; Mukherjee et al., 2017). Findings from the present study supported inclusion of behavioral signs of LS pain in future research studies evaluating relationships between quantitative CT phenotypic traits and early retirement in MWDs.

Mukherjee et al. (2017) recently reported the presence of “funnel” shaped vertebral foramen in a population of young Labrador Retriever MWDs and proposed that this could be an undesirable phenotypic trait for canine athletes because of the potential for impaired arterial perfusion and oxygenation of nerve tissues. The present study also quantified this phenotypic trait in a population of German shepherd MWDs of varying ages by calculating cranial:caudal vertebral foramen area ratio, as well a standardized cranial: caudal vertebral foramen area ratio. All mean ratios within the present study were below 1.0 (i.e., an equal distribution), which indicated that the cranial vertebral foramen was smaller than the caudal vertebral foramen. This interpretation combined with the results of study 2 suggests that funnel-shaped vertebral canals may be a common phenotypic trait in German shepherd MWDs and that the degree of “funneling” may be more pronounced in the L7 vertebra. An unexpected finding was that vertebral foramen area ratio, cranial:caudal vertebral foramen area ratio, and standardized cranial: caudal vertebral foramen area ratio was significantly affected by concurrent disease score. Further studies may be warranted to determine whether MWDs with funnel-shaped vertebral canal phenotypes could be at greater risk for later development of concurrent diseases. If so, it may be worthwhile to screen for this

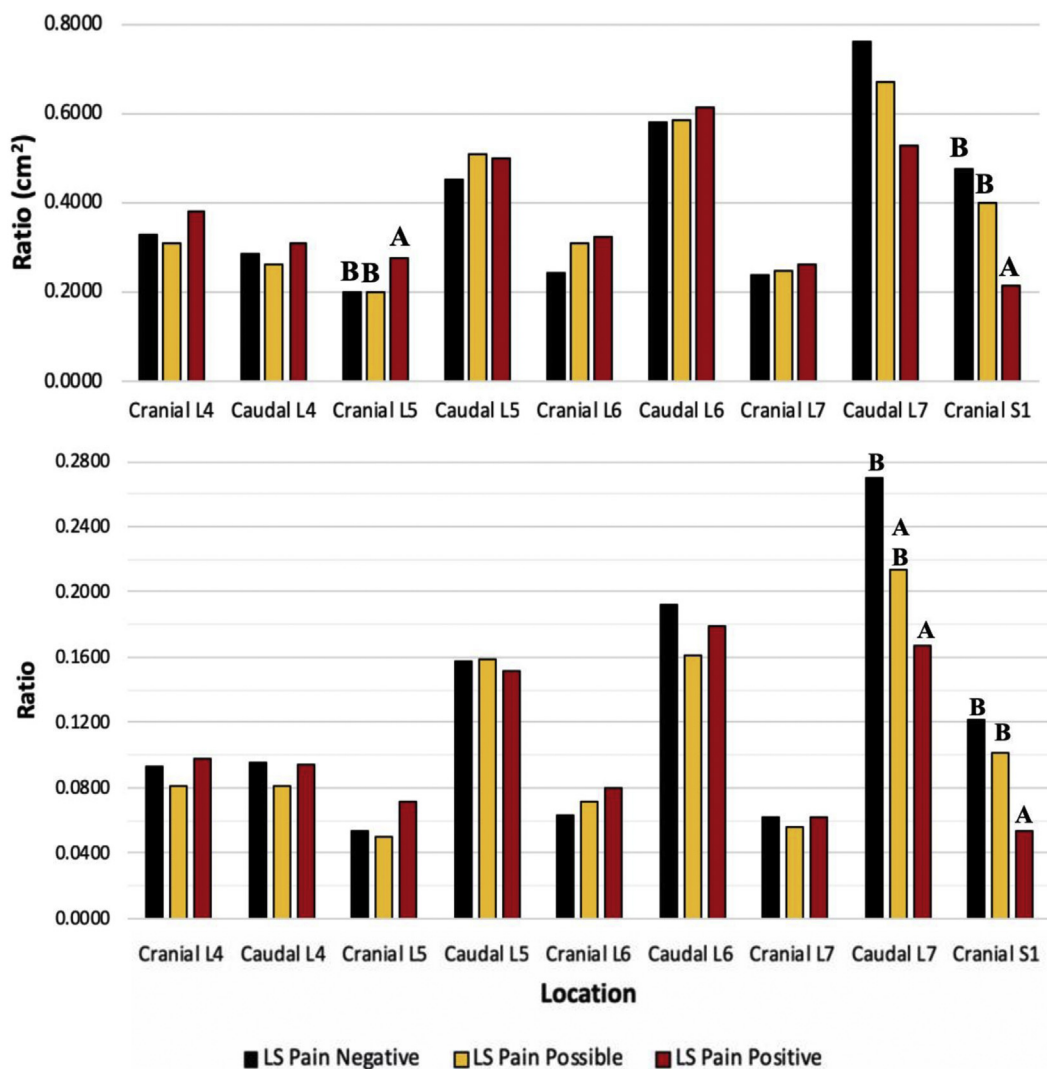


Figure 11. Graphical comparison of vertebral foramen fat area.

phenotype using lateral radiographs of the LS spine at the time of procurement.

Mukherjee et al. (2017) developed a novel methodology to quantify transverse vertebral foramen fat area and found this measurement to be a positive predictor of LS stenosis. Mukherjee and colleagues (2017) found significant differences for vertebral foramen fat area at four vertebral locations, as well as significant differences for vertebral foramen fat area ratio for all vertebral locations. By contrast, results in the present study indicated that the average vertebral foramen fat areas within the cranial L5, cranial L7, and cranial S1 vertebral foramen locations were significantly different between LS pain–positive and LS pain–negative dogs; however, no significant differences were observed within the vertebral foramen fat area ratios of any vertebral foramen location. The differences between studies could be due to breed or age differences in the sampled populations. Nonetheless, the presence of a subjective decline in vertebral foramen fat area in the LS pain–positive groups could suggest that a reduced vertebral foramen fat area could be a risk factor worth exploring in future research studies.

An unexpected finding within the present study was the significantly greater vertebral foramen fat area in the cranial L5 vertebral location for the LS pain–positive dogs versus the LS pain–

negative dogs. Previous studies that have experimentally induced multilevel stenosis in pigs concluded that double-level compression can impair neural function, restrict blood flow between compressed sites, and reduce the increase in blood flow velocity necessary to maintain nerve root function during exercise (Matsui et al., 1992; Baker et al., 1995; Jespersen et al., 1995; Jespersen et al., 1997). Within the present study, it could be possible that the German Shepherd dogs with LS pain had a larger cranial L5 vertebral foramen fat area because of nerve tissue atrophy stemming from compressed sites cranially and caudally; however, this could not be confirmed within this population of dogs because of the lack of vertebral locations above the L4 vertebra being included in all of the imaging studies.

Mukherjee et al. (2017) developed vertebral foramen fat area ratio thresholds that predicted the probability of a vertebral location being LS stenosis positive based on its vertebral foramen fat area ratio value. These vertebral foramen fat area ratio thresholds were used in the present study, and the number of dogs with multilevel stenosis was significantly higher within the LS pain–positive group of the first pain classification when compared with the dogs without LS pain. This finding supports use of this quantitative measure of multilevel stenosis in future studies of risk factors for early retirement in MWDs.

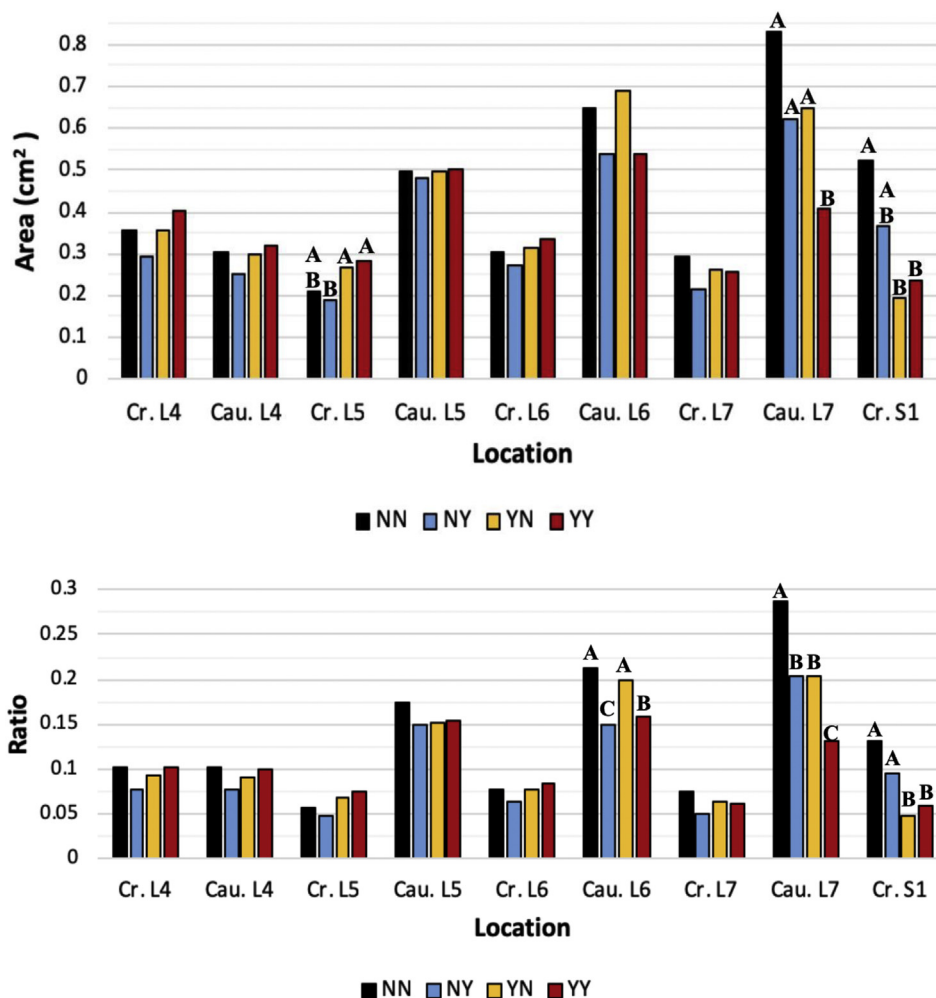


Figure 12. Graphical comparison of vertebral foramen fat area (top) and vertebral foramen fat area ratio (bottom): Third pain classification. Significant differences determined through an ANOVA and pairwise Student's t-tests are denoted by a difference in lettering ($P < 0.05$). "NN", lumbosacral pain negative without behavioral signs of pain; "NY", lumbosacral pain negative with behavioral signs of pain; "YN", lumbosacral pain positive without behavioral signs of pain; "YY", lumbosacral pain positive plus behavioral signs of pain. "Cr.", cranial; "Cau.", caudal.

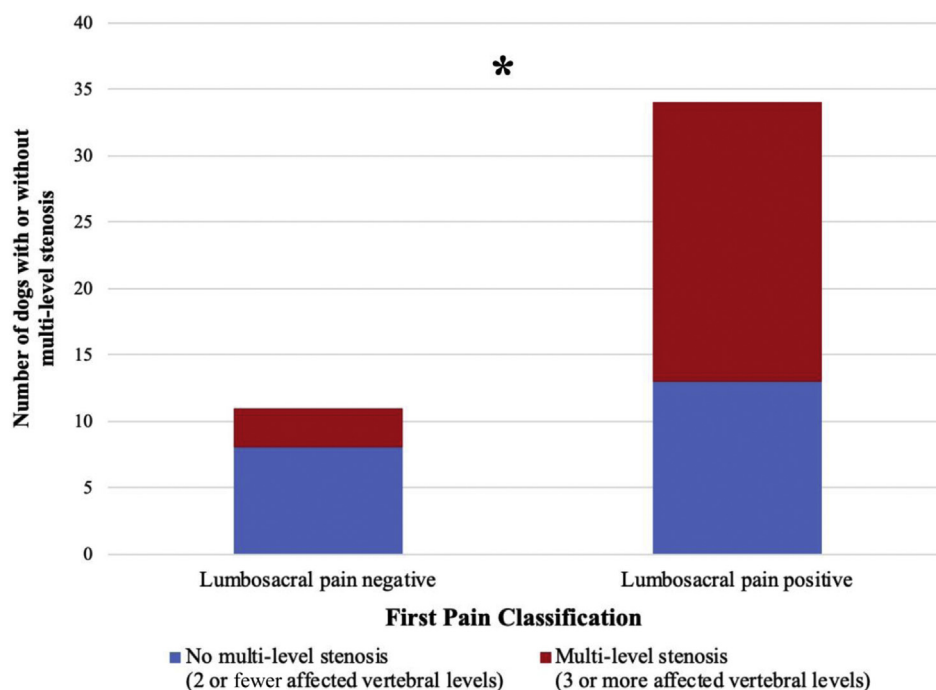


Figure 13. Graphical comparison of multilevel stenosis determined through vertebral foramen fat area ratio thresholds: First pain classification. Significant differences determined through a Fischer's exact test are denoted by an asterisk ($P < 0.05$).

The intention of measuring vertebral canal volume was to propose a new methodology for measuring multilevel stenosis; however, this quantitative measure was not found to be significantly different between any of the LS pain groups. Previous studies conducted by Miao et al. (2013) and Li et al. (2016) in humans concluded that the spinal canal volume of patients with stenosis were significantly lower than the spinal canal volume observed for control patients.

As with study 1, a limitation of study 2 was the use of medical record excerpts rather than complete medical records. A second limitation of the study was the inability to control the positioning of the dogs during their CT scans. The effects of this limitation were minimized as much as possible by using extension scans for all data recording, focusing on static bony vertebral canal measurements, and using multiplanar reformatting to adjust for patient positioning obliquity. Finally, a third limitation of study 2 was the multifactorial nature of behavioral problems, making it difficult to confirm that the recorded behavioral problems were not caused by another concurrent disease. We attempted to minimize this limitation by including concurrent diseases as a covariate in our comparison analyses.

Conclusions

Results of study 1 that did not support the hypothesis include that there was not a significant difference in the percentage of affected dogs with multilevel stenosis between LS pain groups based on current standard, qualitative clinical and CT diagnostic criteria. Other multilevel spinal lesions were also found in similar percentages between LS pain groups, with the exception of multilevel articular process degenerative joint disease. This lesion was present in significantly more dogs with LS pain compared with those without LS pain.

Results within study 2 that supported the first hypothesis that the severity of quantitative CT characteristics would be significantly different between LS pain groups includes the finding that dogs

with LS pain were significantly more affected by multilevel stenosis than dogs without LS pain when characterized by previously published quantitative vertebral foramen fat area ratio threshold values. Regarding transverse vertebral foramen fat area, the cranial L7 and cranial S1 vertebral locations were significantly different between LS pain groups for the first and second pain classifications. The vertebral foramen fat area of the cranial L5, caudal L7, and cranial S1 vertebral locations were significantly different between LS pain groups in the third classification. An unexpected finding was that the vertebral foramen fat area of cranial L5 was larger in the LS pain positive group; however, this finding did not occur in any other vertebral level. Significant differences between groups only occurred for the vertebral foramen fat area ratio for the second (i.e. caudal L7; cranial S1) and third (caudal L6; caudal L7; cranial S1) pain classification systems. When accounting for concurrent disease score, the cranial:caudal vertebral foramen area ratio was significantly lower in the L7 vertebral level, followed by the L6 vertebra, then the L4 and L5 vertebral levels in all LS groups. This result suggests that a funnel-shaped vertebral canal may be present in German shepherd MWDs without detectable LS pain. A result of study 2 that did not support the first hypothesis includes the finding that the vertebral canal volume was not significantly different between any of the LS pain groups within the three pain classification systems.

Results within study 2 that did not support the second hypothesis that concurrent diseases would not affect the quantitative CT measurements include that the concurrent disease score significantly affected the vertebral foramen area ratio, cranial:caudal vertebral foramen area ratio, and the standardized cranial:caudal vertebral foramen area ratio. The clinical significance of the effects of concurrent diseases on these quantitative measurements is currently unknown.

Future studies should explore the effects of infusing pain-relieving treatments into articular process joints as a method for localizing LS pain in military dogs. Authors recommend that the L4 and L5 vertebrae be included in CT scans for dogs presenting with

LS pain so that the multilevel stenosis phenotype can be more readily assessed. Researchers should also explore whether multilevel stenosis is caused by genetic or developmental causes and determine if the clinical or behavioral signs of LS pain are more severe within dogs with multilevel stenosis when compared with those with single-level stenosis. Future studies should consider exploring the effects of concurrent diseases on behavioral problems in dogs with quantitative phenotypic characteristics of vertebral canal stenosis. Finally, researchers should consider using quantitative CT phenotypic traits and LS pain classifications that include behavioral problems for future studies evaluating causes of early retirement in German shepherd MWDs.

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Conflicts of interest statement

The authors have no conflicts of interest to disclose.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jveb.2020.05.011>.

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