

Computed Tomographic Studies of Characteristics of Selected Canine Lymph Nodes Relevant to
Staging and Treatment of Solid Tumours

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By

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1. Introduction

Tumour staging is used for prognostication and treatment planning, and is based on the TNM (tumour, node, metastasis) system. In dogs with solid tumours, the location of regional lymph nodes and patterns of lymphatic drainage are critical to staging and radiation treatment. The desired radiation treatment regime is one which includes all diseased regions in the radiation field without irradiation of normal tissues.

The overall objective of the three computed tomographic (CT) studies included in this manuscript-style thesis was to provide information which could improve clinical decisions regarding the staging and radiation treatment of pelvic limb and caudal abdominal tumours in dogs. Specific objectives of this body of research were to describe the lymphatic drainage of the canine pelvic limb, characteristics of normal popliteal lymph nodes, and the location and CT characteristics of presumed normal medial iliac lymph nodes.

Study of the popliteal and medial iliac lymph nodes were performed because they can both be considered nodal stations of the pelvic limb and caudal body of the dog. The popliteal lymph node drains lymph from the limb distal to it. The medial iliac lymph node is known to receive efferent lymph flow from the popliteal lymph node, as well as drainage directly from tissues of the caudal abdomen, pelvic canal, and perineal region. Tumours in body areas that drain to the popliteal and medial iliac lymph nodes that are commonly treated with radiation therapy include anal sac gland adenocarcinoma, mast cell tumours, and squamous cell carcinomas.

Literature reviews indicated there was limited evidence about lymphatic flow from the popliteal lymph node in dogs. Available information comes from the work of Baum who performed anatomical and lymphographic studies in the early 1900s, and Pflug and Schacher who performed independent lymphographic studies in the 1960s and 1970s.^{1,2,3} These studies, including only a few dogs, demonstrated lymph flow from the popliteal lymph node to the

medial iliac lymph nodes.^{1,2,3} For this reason, clinical practice is irradiation of the medial iliac lymph nodes when malignant cells are detected or suspected.^{4,5}

A widely referenced anatomy text reported that the superficial inguinal lymph node received efferent flow from the popliteal lymph node.⁶ For this reason, clinical practice is to include the superficial inguinal lymph nodes in the treatment field of radiation therapy. However, three previously published lymphography studies did not report any flow from the popliteal lymph node to the superficial inguinal lymph nodes.^{1,2,3}

The study described in Chapter 2, *Patterns of Lymphatic Drainage of the Popliteal Lymph Node in Dogs*, was conducted to determine flow patterns from the popliteal lymph node in dogs, using a larger sample (N = 50) than had previously been reported in the literature. It was designed to assess flow from the popliteal lymph nodes to the medial iliac lymph nodes, superficial inguinal lymph nodes and other lymph nodes in the pelvic canal. The results with respect to the medial iliac, superficial inguinal, internal iliac and sacral lymph nodes can help determine whether treatment of these lymph nodes is indicated.

Size is a widely accepted imaging method for discriminating between normal and pathological lymph nodes.^{7,8} Factors that have previously been reported to be associated with lymph node size include the dog's age and body weight.^{9,10} Secondary objectives of the lymphographic study were to describe computed tomographic and ultrasonographic characteristics of the popliteal lymph node. This information can contribute to assessment of whether the popliteal nodes are enlarged and hence can have staging and treatment implications.

Since this study showed that all the dogs had flow from the popliteal lymph node to the medial iliac lymph node, it confirmed that the medial iliac lymph node should be included in the radiation field. However, in cases where 3D cross-sectional imaging is not available, the normal location of the medial iliac lymph nodes relative to something that can be visualized on 2D x-ray images or palpated is required to accurately include the medial iliac lymph nodes in the radiation field. Very little of this information has been reported in the literature. The specific objective of the study described in Chapter 3, *Location of Normal-Sized Medial Iliac Lymph Nodes Relative to Lumbar Vertebrae in 100 Dogs*, was to provide that information.

In cases where 3D cross-sectional imaging is available, a baseline of CT characteristics of normal medial iliac lymph nodes with no known disease would be useful in assessing whether there is evidence of disease in the medial iliac lymph nodes on CT examination. One published

study on the CT characteristics of the abdominal lymph nodes reported the range and median dimensions of 42 lymph nodes measured in 19 dogs.¹¹ Another paper reported the mean dimensions of normal and abnormal medial iliac lymph nodes in 12 dogs, but all of these patients had been diagnosed with anal sac gland adenocarcinoma.¹² The specific objective of the study described in Chapter 4, *Characteristics of Presumed Normal Canine Medial Iliac Lymph Nodes on Computed Tomographic Examination*, was to provide a normal reference range for the size and attenuation of the medial iliac lymph nodes of 200 dogs without suspicion of disease in the lymph nodes. This reference range could then be used to guide clinical decisions to sample or treat medial iliac lymph nodes that are enlarged.

In summary, the purpose of the body of research reported in this thesis was to provide clinically relevant information for the staging and treatment of pelvic limb and caudal abdominal tumours in dogs. The results provide essential information to clinicians about lymph nodes and drainage patterns in the canine pelvic limb, and the location and CT characteristics of presumed normal medial iliac lymph nodes. This information is expected to help guide clinicians in decision-making with respect to sampling lymph nodes in the drainage area of the tumour and determining appropriate radiation therapy.

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2. Patterns of Lymphatic Drainage of the Popliteal Lymph Node in Dogs

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At the time of preparation of this thesis, this manuscript has been submitted to the journal *Veterinary Radiology & Ultrasound* and is being considered for publication.

This study was underway when I began my program. My contributions to this study included: recruiting participants, data accrual and interpretation, assistance in literature review and manuscript writing and editing.

2.1 Abstract

Staging and therapeutic planning for dogs with malignant disease in the popliteal lymph node are based on the expected patterns of lymphatic drainage from the node. The medial iliac lymph nodes are known to receive efferent lymph from the popliteal lymph node; however, an accessory popliteal efferent pathway with direct connection to the sacral lymph nodes has also been less frequently reported. The primary objective of this anatomic study was to describe the frequency of various patterns of lymphatic drainage of the popliteal lymph node. Fifty adult dogs with no known disease of the lymphatic system underwent computed tomographic lymphography after ultrasound-guided, percutaneous injection of 350mg/ml iohexol into a popliteal lymph node. In all 50 dogs, the popliteal lymph node drained directly to the ipsilateral medial iliac lymph node through multiple lymphatic vessels that coursed along the medial thigh. In 26% (13/50) of dogs, efferent vessels also drained from the popliteal lymph node directly to the internal iliac (previously called the hypogastric) and/or sacral lymph nodes, coursing laterally through the gluteal region and passing over the dorsal aspect of the pelvis. Lymphatic connections between the right and left medial iliac and right and left internal iliac lymph nodes were found. Based on our findings, the internal iliac and sacral nodes should be considered when staging or planning therapy for dogs with malignant disease in the popliteal lymph node.

2.2 Introduction

The superficial popliteal node in the dog is a constant, usually single, lymph node located caudal to the stifle joint between the medial border of the biceps femoris muscle and the lateral border of the semitendinosus muscle.^{1,2} The afferent lymph vessels drain the hind limb distal to the node, including the skin of the caudal half of the lateral stifle and lower limb, the skin of the lateral, cranial and caudal tarsus, and the skin of the metatarsus and phalanges.² Lymph also drains to the popliteal lymph node from the tibia and fibula, the bones of the tarsal, metatarsal and phalangeal regions, the tarsal and phalangeal joints, and muscles of the lower hind limb.² Lymph nodes that receive efferent lymphatics from the popliteal lymph node have been reported to include the medial iliac lymph nodes, the superficial inguinal lymph nodes, the internal iliac (formerly called the hypogastric) lymph nodes, as well as the inconstant distal femoral and external iliac lymph nodes, when present.¹⁻⁶ The patterns of lymphatic drainage from the popliteal lymph node become important during staging and therapeutic planning for dogs that have malignant disease in the node.

The medial iliac lymph nodes receive efferent lymphatics from the popliteal lymph node in 100% of dogs.^{2,3,5} The efferent lymphatic vessels from the popliteal node follow the lateral saphenous vein as it passes medially and deeply to join the popliteal vein at mid-thigh level, then follow the popliteal vein as it passes through the inguinal region and becomes the external iliac vein, and then follow the iliac veins to drain into the medial iliac lymph node.^{3,5} When the distal femoral lymph node or external iliac lymph node (formerly called the iliofemoral or deep inguinal lymph node) are present, one of the lymphatic vessels following this pathway usually enters them first.² The lymphatic vessels leaving the distal femoral and external iliac lymph node drain into the medial iliac lymph nodes.

The likelihood of lymph drainage from the popliteal lymph node to the superficial inguinal lymph nodes is less clear. Three comprehensive lymphography studies of pelvic limb lymphatics in the dog found no drainage of lymph from the popliteal lymph nodes to the superficial inguinal lymph nodes.^{2,3,5} A very superficial system of lymphatics starting just above the level of the hock and connecting to the superficial inguinal lymph node is described; however, this system of lymphatics did not connect with the popliteal lymph node.³ In contrast to these findings, a recognized canine anatomy textbook states that “the superficial inguinal lymph

nodes receive efferent vessels from the popliteal lymph node and thus serve as one of the nodal stations for the whole pelvic limb”.¹

Direct drainage of lymph from the popliteal lymph node to the sacral and internal iliac lymph nodes has been reported to occur infrequently.^{2,3,5} The efferent lymphatics from the popliteal lymph node course along the deep surface of the semitendinosus muscle parallel to the sciatic nerve, then course laterally through the gluteal region and pass dorsally to the pelvic bones to enter the pelvis and connect to the sacral and internal iliac lymph nodes. Baum reported this drainage pattern in 1 dog, while Schacher et al. reported it in 8% (2/26) and 11% (2/19) of dogs in two studies, calling it the accessory popliteal efferent pathway.^{2,4,5} The sacral nodes that these vessels can drain to are present only about half the time, and are small nodes located caudal to the internal iliac lymph nodes, ventral to the body of the sacrum.² The sacral nodes drain to the internal iliac lymph nodes, and are not sharply differentiated from the more constant internal iliac lymph nodes.¹

The objective of this anatomic study was to describe the frequency of various patterns of lymphatic drainage of the popliteal lymph node in clinically normal dogs, using computed tomographic (CT) direct lymphography after ultrasound-guided, percutaneous injection of water-soluble contrast material into the node. A second objective was to describe the ultrasound and CT characteristics of normal popliteal lymph nodes.

2.3 Materials and Methods

The study protocol was approved by the University of Saskatchewan's Animal Research Ethics Board (Animal Use Protocol Number 20120330). Computed tomographic scans of 50 adult dogs were acquired before and after ultrasound-guided, percutaneous contrast injection into a single popliteal lymph node. Dogs were selected from the college teaching colony, other research projects, and client-owned animals scheduled to undergo CT examination. Inclusion criteria included no known disease affecting the lymph nodes, no known medical contraindication to sedation or general anesthesia, and, in the case of client-owned animals, consent from the owner and the primary clinician.

Breed, weight and gender were recorded for each dog. Dogs were imaged under sedation using intravenous injection of 5µg/kg dexmedetomidine (Dexdomitor, Zoetis, Kirkland, QC, Canada) and 0.2mg/kg butorphanol (Torbugesic, Zoetis, Kirkland, QC, Canada), with the addition of intravenous 0.01mg/kg acepromazine (Acevet 25 Injectable, Vetoquinol, Lavaltrie, QC, Canada) in some cases, or under a different sedation protocol or general anesthesia if required by another researcher or for a clinical patient.

Dogs were positioned in dorsal recumbency with hind limbs extended. A pre-contrast CT scan extending from the thoracolumbar junction to the lower limb distal to the popliteal lymph node was acquired, using a 16-slice scanner (Acquilion 16, Toshiba America Medical Systems, Tustin, CA). The following scan parameters were used for all scans: 120 kVp, 250 mA, tube rotation time 0.5 s, and 512 x 512 matrix dimensions. Slice thickness was 1 mm, or 0.8 mm when requested by another researcher, or by a radiologist for clinical patients.

Dogs were randomized to receive contrast injection into the right (n = 25) or left (n = 25) popliteal lymph node. A board-certified veterinary radiologist or radiology resident located the popliteal node using ultrasound examination, and the width, height and length of the node were measured. The maximum width and height were recorded with the transducer in transverse orientation and maximum length with the transducer in longitudinal orientation. The short-to-long axis of the lymph node was calculated using the shortest transverse measurement divided by length as previously described.⁷ Ultrasound was performed using a curved array high frequency ultrasound transducer (8-5 multi frequency curved array probe, Philips iU22, Philips Medical

Systems, Bothell, WA) or a convex array high frequency ultrasound transducer (11MC4 multifrequency curved array probe, Aplio 300, Toshiba America Medical Systems, Tustin, CA).

A 1.0 or 1.5 inch, 22 gauge needle was inserted into the node using ultrasound guidance, and 350mg/ml iohexol was manually injected through an intravenous extension set over 5 minutes, with continued visualization of the node during the injection. A dose of 1ml/kg of iohexol was used for the first 7 dogs, and thereafter a dose of 1ml/kg iohexol up to a maximum volume of 10 ml was used, and the infusion was stopped if perinodal leakage was seen. The change in contrast dose was made due to leakage of contrast around the node noted with the first 7 dogs. The lymph node was massaged for 1 minute, and a 5 minute post-contrast injection CT scan was acquired. The timing of the post-contrast scan was based on findings in previous studies.^{8,9}

Pre- and post-contrast CT scans were used to examine the course of efferent lymphatic vessels, and to classify contrast enhancement (yes/no) of ipsilateral and contralateral medial iliac lymph nodes, superficial inguinal lymph nodes, internal iliac, sacral, distal femoral and external iliac lymph nodes, and of any other nodes visualized. Lymph nodes were classified as internal iliac if they were located caudal to the medial iliac lymph nodes and ventral to the seventh lumbar vertebra, and as sacral if they were located ventral to the sacrum.¹

Pre-contrast CT scans were used to collect the width, height, length and x-ray attenuation of the right and left popliteal lymph nodes, and the number of popliteal nodes on each side. Axial images were examined to subjectively determine the widest portion of each popliteal lymph node, and width was defined as the greatest diameter of the lymph node at that location. Height was defined as the diameter perpendicular to the width measurement on the same slice. Length was obtained by multiplying the slice thickness by the number of axial slices on which the lymph node was visible. Lymph node volume was calculated based on the formula for the volume of an ellipsoid: $\pi/6 \times \text{width} \times \text{height} \times \text{length}$. X-ray attenuation in Hounsfield units (HU) was measured using the picture archiving and communication system elliptical region-of-interest tool, using a region of interest made as large as possible on the axial slice used for width and height measurement. If more than one popliteal lymph node was present on the right or left side, the largest lymph node on that side was used to measure size and x-ray attenuation.

Statistical tests were selected and completed by an analytical epidemiologist using a commercial statistical software (STATA/SE version 14.1 for Windows, StataCorp, College

Station, TX). P-values ≤ 0.05 were considered statistically significant. Descriptive statistics (mean, standard deviation, median, and the 2.5th and 97.5th percentiles) were calculated for lymph node size and attenuation measurements. Body weight, patient age and side of node (right versus left) were examined as potential predictors of lymph node size on CT with mixed linear regression accounting for repeated measures on the same dog (right and left lymph node). These potential predictors were not examined for lymph node size measured with ultrasound due to the low number of measurements available (less than 50). Body weight and patient age were examined as they have been previously reported to be associated with lymph node size.¹⁰ The side of node was examined to determine if the contralateral node could be used for comparison if a node is suspected to be enlarged and if the disease process would only be expected to affect one side (e.g. a solid tumor draining to a node).

We also examined whether lymph node size (width, height, length, volume and short-to-long axis ratio) differed based on imaging modality, to see if direct comparisons could be made between measurements made with CT and with ultrasound. The significance of differences in width, height and length of nodes measured using both CT and ultrasound were summarized using paired t-tests. Agreement between node volume and then the short-to-long axis ratio as measured by ultrasound and CT was examined using Lin's concordance correlation coefficient. The short-to-long axis ratio was calculated using the shortest transverse measurement divided by length for ultrasound and the width divided by length for CT.

2.4 Results

Twenty-five dogs were clinically healthy research dogs, 17 were healthy dogs in the college teaching colony, and 8 dogs were client-owned. Of the client-owned dogs, 4 were healthy and volunteered for the study by their owner. The remaining 4 underwent CT scanning for lipoma, congenital urinary incontinence, thyroid carcinoma and chylothorax. The study period extended from October 2012 to December 2016.

Ten of the research dogs did not have a date of birth available, but were known to be between 1 and 8 years of age. The median age of the remaining dogs was 3.2 years (range, 1.0 to 11.6 years). The median body weight was 19.8kg (range, 7.8 – 49.0kg). There were 15 neutered males, 10 intact males, 15 neutered females and 10 intact females. Breeds included mixed (n = 31), Beagle (n = 15), and 1 each of Labrador Retriever, Bull Mastiff, Bernese Mountain Dog and Whippet.

Dogs were imaged in dorsal recumbency, with the exception of 1 client-owned dog imaged in ventral recumbency. Slice thickness was 1.0 mm (n = 44 dogs) and 0.8 mm (n = 6 dogs). Ultrasound measurements were available for the right popliteal lymph node in 20 dogs and the left popliteal lymph node in 23 dogs (measurements were not recorded for the first 7 dogs in the study). CT measurements were available for the right popliteal node in 47 dogs and the left popliteal node in 48 dogs (measurements were not available for 5 nodes as the CT scan did not include the entire node).

The first 7 dogs received 1ml/kg iohexol 350mg/ml intranodally, with a median volume of 9.2 ml (range, 7.8ml to 26.6ml). The remaining dogs, with the exception of 1 dog, received the same dose up to a maximum volume of 10 ml, with discontinuation of injection if leakage around the node was seen ultrasonographically. The median volume of contrast for these 42 dogs was 7.4 ml (range, 2.8 ml to 10 ml), and injection was stopped in 24 dogs due to perinodal leakage. One dog received 20 ml of contrast for lymphography to assess a chylothorax.

The presence of contrast in lymph nodes receiving lymph from the popliteal lymph node is described in Table 1. In all dogs, the efferent lymphatic vessels coursed medially from the proximal end of the lymph node along the medial thigh to the inguinal region, and then dorsally to the medial iliac lymph node (Figure 1). In 4% (2/50) of dogs, the lymphatic vessels first

entered a distal femoral lymph node located on the medial side of the thigh at the distal end of the femoral canal (Figure 2).

In 10% (5/50) of dogs, contrast material was also seen in the ipsilateral superficial inguinal lymph node. All these dogs were intact (4 female, 1 male), and all had leakage of contrast material in the tissues immediately surrounding the injected popliteal lymph node. A direct connection between the superficial inguinal lymph node and the vessels draining to the medial iliac lymph node was not seen, although the vessels coursed in close proximity to the superficial inguinal lymph node. In 2 of these dogs, a single efferent lymphatic vessel that appeared to originate at the contrast material outside the popliteal lymph node coursed superficially along the medial thigh to the superficial inguinal lymph node (Figure 3).

In 26% (13/50) of dogs, efferent lymphatics from the popliteal lymph node coursed proximally and laterally along the deep surface of the semitendinosus muscle, then passed dorsally to the pelvic bones and connected to internal iliac and/or sacral lymph node(s) (Figure 4). Of these dogs, 100% (13/13) had contrast present in an ipsilateral internal iliac and/or sacral lymph node, and 62% (8/13) had contrast present in a contralateral internal iliac and/or sacral lymph node. In 1 dog, the lymphatic vessels first entered a lymph node located on the dorsal and lateral aspect of the rectum (Figure 5).

Of the 37 dogs lacking a direct efferent pathway to the internal iliac/sacral nodes, 27% (10/37) had contrast present in the ipsilateral and contralateral ($n = 7$) or ipsilateral ($n = 3$) internal iliac nodes. In 4 of these dogs, contrast was visible in lymphatic vessels connecting the medial iliac lymph node(s) and internal iliac lymph node(s) of the same side. Of the 13 dogs with contrast visible in both internal iliac nodes, contrast in vessels connecting the nodes was present in 85% (11/13) (Figure 6). As well, 2 dogs with contrast in only the ipsilateral medial iliac lymph node had contrast in both the ipsilateral and contralateral internal iliac/sacral lymph nodes, supporting a connection between the left and right internal iliac lymph nodes.

Characteristics of the popliteal lymph nodes on ultrasound and CT are presented in Tables 2 and 3. Short-to-long axis ratio is reported for ultrasound and length-to-width ratio for CT to facilitate comparison with reported values.^{11,12} Popliteal lymph nodes were doubled bilaterally on 1 dog, doubled only on the left side in 4 dogs, and doubled only on the right side in 3 dogs.

The CT-measured width, height and length of the popliteal lymph node increased with increasing body weight ($p \leq 0.001$). The width, height and length of the node decreased with increasing patient age ($p \leq 0.001$, $p \leq 0.001$, and $p = 0.032$). There was no difference in CT width, height and length between the right and left side of the body ($p = 0.35$, $p = 0.33$ and $p = 0.19$).

The widths (difference = 1.33 mm, 95% C.I. 0.34 to 2.34, $p = 0.01$) and lengths (difference = 5.39 mm, 95% C.I. 3.83 to 6.74, $p < 0.001$) of the nodes measured by CT were significantly greater than the ultrasound measurements. However, the height of the nodes measured by CT was not significantly greater than that measured by ultrasound (difference = 0.67 mm, 95% C.I. -0.04 to 1.39, $p = 0.06$). There was moderate agreement between the volume of the popliteal lymph node measured by ultrasound and the volume of the node measured by CT (concordance correlation coefficient = 0.57). The agreement between short-to-long axis ratio as measured by ultrasound and CT was excellent (concordance correlation coefficient = 0.92).¹³

2.5 Discussion

The direct drainage from the popliteal lymph node to the medial iliac lymph node(s) in all the dogs in this study is consistent with previous studies.^{2,3,5} The distal femoral lymph node has been reported to be present in 5-13% of dogs, often only on one side, and this is also consistent with our finding of contrast in a femoral lymph node in 4% (2/50) of dogs.^{2,4,5} Although the distal femoral lymph node is inconstant, it should be considered when staging a dog with neoplastic disease in the popliteal lymph node, given the direct connection between these nodes. The external iliac lymph node has been reported to be present in 36% of dogs, often only on one side.^{2,4,5} We did not identify this node in any dog, possibly due to its small size.

We identified contrast in the ipsilateral superficial inguinal lymph node in 10% of dogs. Previous studies have found no drainage from the popliteal lymph node to the superficial inguinal lymph nodes.^{2,3,5} A superficial system of lymphatic vessels in the hind limb draining to the superficial inguinal lymph node has been described.³ The possibility of contrast material that leaked outside of the popliteal lymph node draining via this superficial system to the superficial inguinal lymph node, rather than efferent vessels passing from the popliteal node to the superficial inguinal lymph node, must be considered. Additional study using smaller volumes of contrast material to avoid perinodal leakage would be needed to confirm drainage from the popliteal node to the superficial inguinal node.

Efferent vessels from the popliteal lymph node entering the pelvis from the dorsal surface and draining directly to the internal iliac and/or sacral lymph node(s) was seen in 26% of dogs in our study. Given this drainage pattern, the internal iliac and sacral nodes should be considered in staging and therapeutic decisions when the popliteal lymph node contains neoplastic disease.

Our findings indicate that there are lymph vessels connecting the right and left medial iliac lymph nodes, as previously described by Baum, and also connecting the right and left internal iliac lymph nodes.² We also found that contrast flowed caudally from the medial iliac lymph node to the internal iliac lymph node. This retrograde flow may have been a result of the increased pressure in the lymphatic system caused by contrast injection, rather than representing direction of flow that would occur in the normal dog.

To the authors' knowledge, characteristics of the normal popliteal lymph nodes on ultrasound and CT imaging have not been described. While the number of nodes examined in

this study does not support recommending a normal reference range, our findings could be used to raise the level of suspicion when disease is suspected. As previously reported for lymph nodes other than the popliteal lymph node, node size decreased with age and with decreasing body weight.^{10,14} We found that this also held true for the popliteal lymph nodes. The lack of difference between the size of the right and left popliteal nodes in individual dogs suggests that the size of the contralateral node could be used to help decide if a popliteal node is enlarged. The CT length-to-width ratio for normal nodes has been suggested to be greater than or equal to 2, however, based on our 2.5th and 97.5th percentiles this value may be lower for popliteal lymph nodes.

The differences between ultrasound and CT in node width, length and volume measurements were likely due to differences in how measurements were made and in planes of image acquisition. Lymph node length on ultrasound was measured directly, but for CT measurement the number of CT slices with node visible was multiplied by the slice thickness. Because it was not possible to position the long axis of the node in the cranial to caudal direction, the transverse CT images would have presented an oblique section. During ultrasound, however, the user could position the transducer to obtain true short-axis measurements. The agreement between the CT and ultrasound ratios of longitudinal and transverse diameter suggests that, despite the differences in absolute values of node size, the ratios of longitudinal and transverse diameters reported for one modality could be applied to the other. Given the low numbers of nodes examined in this study, additional work is needed to confirm this finding.

In conclusion, in addition to direct drainage from the popliteal lymph node to the medial iliac lymph node, a second drainage pathway from the popliteal lymph node to the internal iliac/sacral lymph nodes exists in 26% of dogs. This accessory popliteal efferent pathway should be considered when staging and planning therapy for dogs that have malignant disease in the popliteal lymph node.

^aGemini TF Big Bore System, Philips Medical System, Cleveland, OH.

^bAcquilion 16, Toshiba America Medical Systems, Tustin, CA.

2.6 References

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2.7 Tables

Table 2.7.1. Percentage of Lymph Nodes with Contrast Uptake after Percutaneous Injection of Iohexol into a Popliteal Lymph Node in 50 Dogs		
	% OF NODES WITH CONTRAST UPTAKE	
	IPSILATERAL	CONTRALATERAL
Medial Iliac Lymph Node	100% (50/50)	48% (24/50)
Superficial Inguinal Lymph Node	10% (5/50)	0%
Internal Iliac Lymph Node	44% (22/50)	26% (13/50)
Sacral Lymph Node	24% (12/50)	10% (5/50)

Table 2.7.2. Computed Tomographic Characteristics of 95 Presumed Normal Popliteal Lymph Nodes in 50 Dogs					
	MEAN	SD^a	MEDIAN	2.5th PCTL^b	97.5th PCTL
Width (mm)	9.9	3.4	9.5	4.5	17
Height (mm)	7.1	2.6	6.8	3.2	12.7
Length (mm)	18.6	6.2	18.0	8.0	32.0
Length-to-width ratio	2.0	0.5	1.9	1.1	3.1
Attenuation (Hounsfield units)	30.7	6.4	30.9	18.7	43.3
Volume (mm ³)	877	802	575	74	2997

^aStandard Deviation

^bPercentile

Table 2.7.3. Ultrasound Characteristics of 43 Presumed Normal Popliteal Lymph Nodes in 50 Dogs					
	MEAN	SD^a	MEDIAN	2.5th PCTL^b	97.5th PCTL
Width (mm)	8.6	2.9	8.6	3.9	14.7
Height (mm)	6.6	2.9	6.0	2.4	11.9
Length (mm)	14.0	5.7	13.0	5.9	25.5
Short-to-long axis ratio	0.5	0.2	0.5	0.25	1.0
Volume (mm ³)	510	475	358	47	1660

^aStandard Deviation

^bPercentile

2.8 Figures

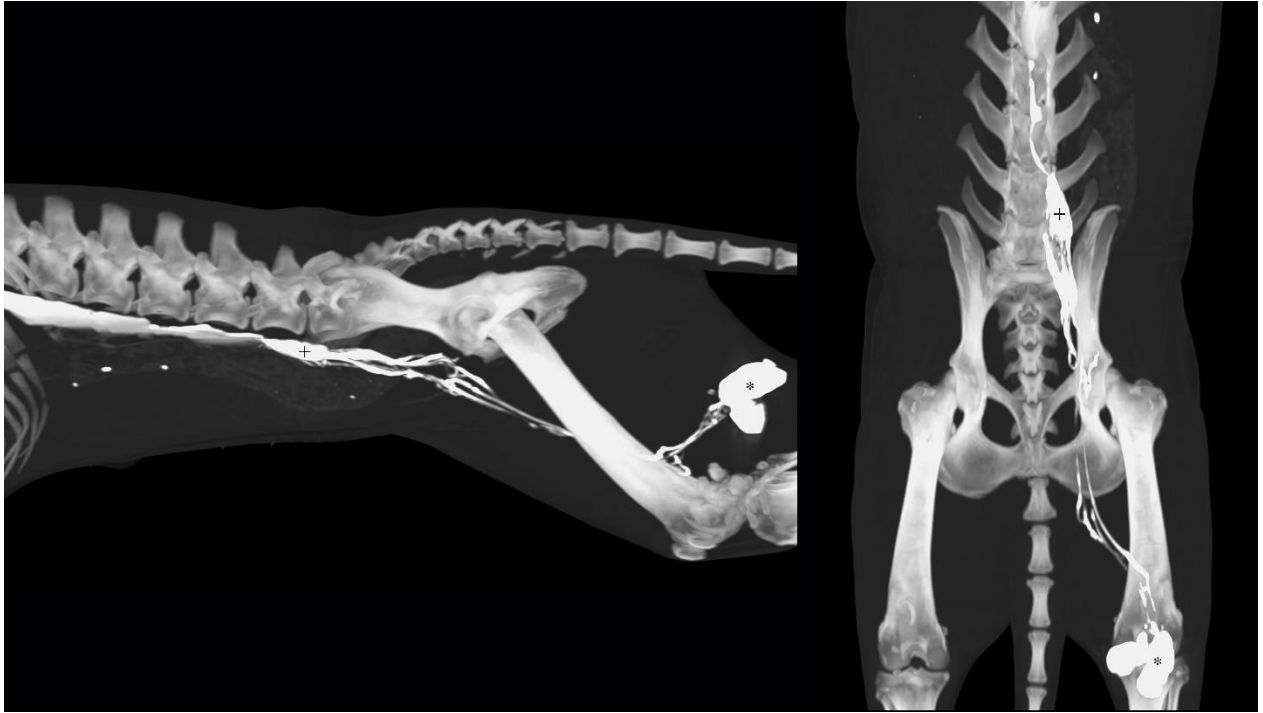


Figure 2.8.1. Left lateral (left) and ventrodorsal (right) maximum intensity projections of a dog 5 minutes after injection of 10 ml of 350 mgI/ml iohexol into the left popliteal lymph node. Lymphatic vessels drain from the popliteal lymph node (*) along the medial hind leg to the ipsilateral medial iliac lymph node (+).

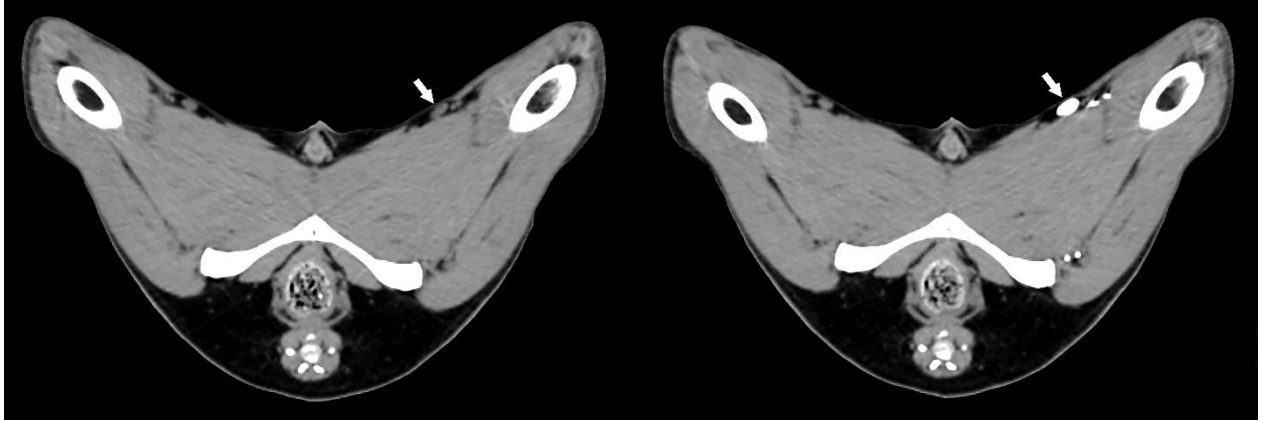


Figure 2.8.2. Transverse computed tomographic images pre- (left) and 5 minutes post-injection (right) of 5.2 ml of 350 mgI/ml iohexol into the left popliteal lymph node. The white arrows indicate the left distal femoral lymph node on the pre-contrast (left) and post-contrast (right) image, and the lymphatic vessels draining to this node are visible just to the right of the node.

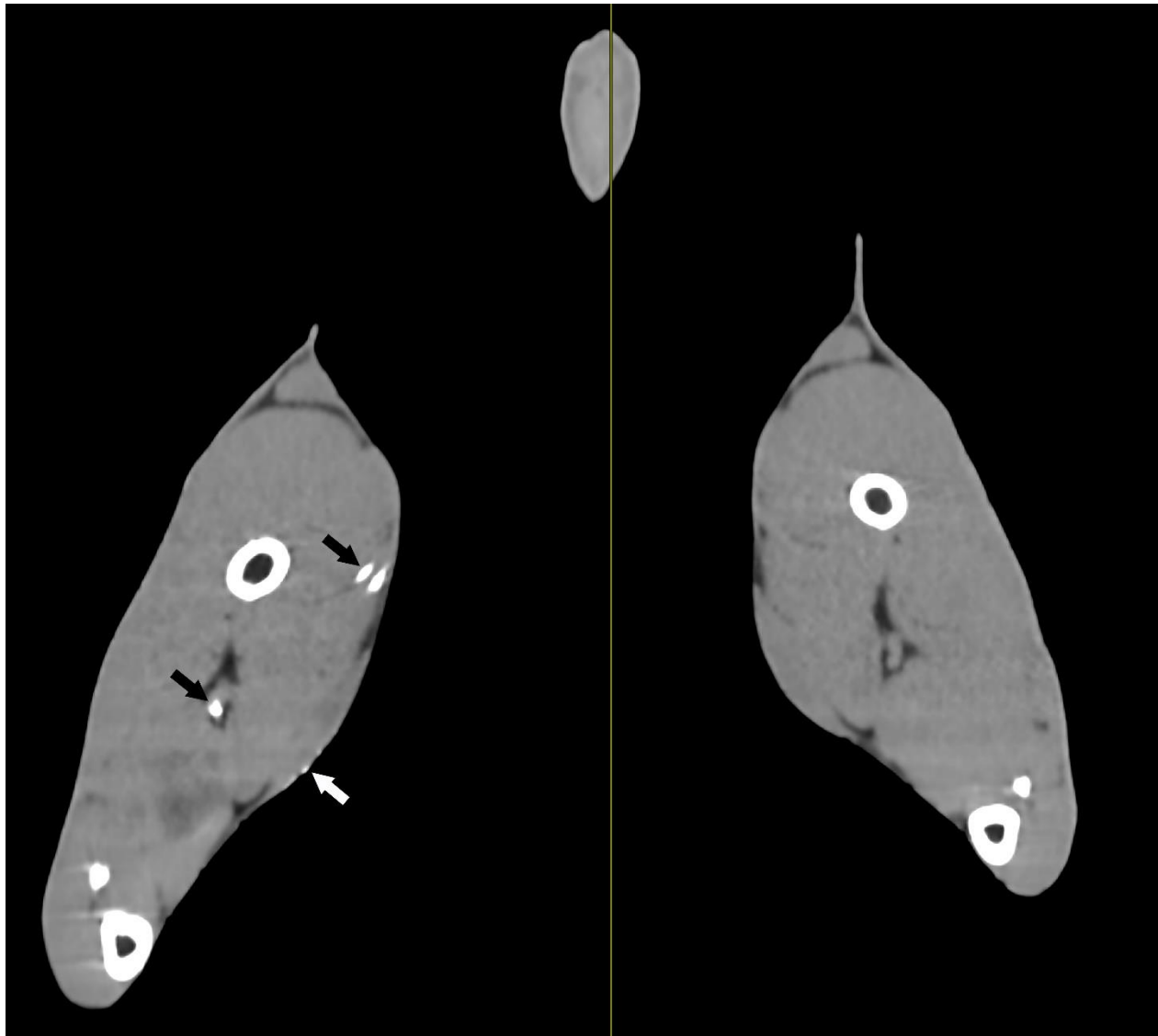


Figure 2.8.3. Coronal plane computed tomographic image of a dog 5 minutes post-injection of 10 ml of 350mgI/ml iohexol into the right popliteal lymph node. A contrast-filled superficial lymphatic vessel that originated at contrast injected under the skin and coursed to the ipsilateral superficial inguinal lymph node is visible on the medial aspect of the hind limb (white arrow). The black arrows indicate lymphatic vessels passing from the right popliteal lymph node to the medial iliac lymph node.

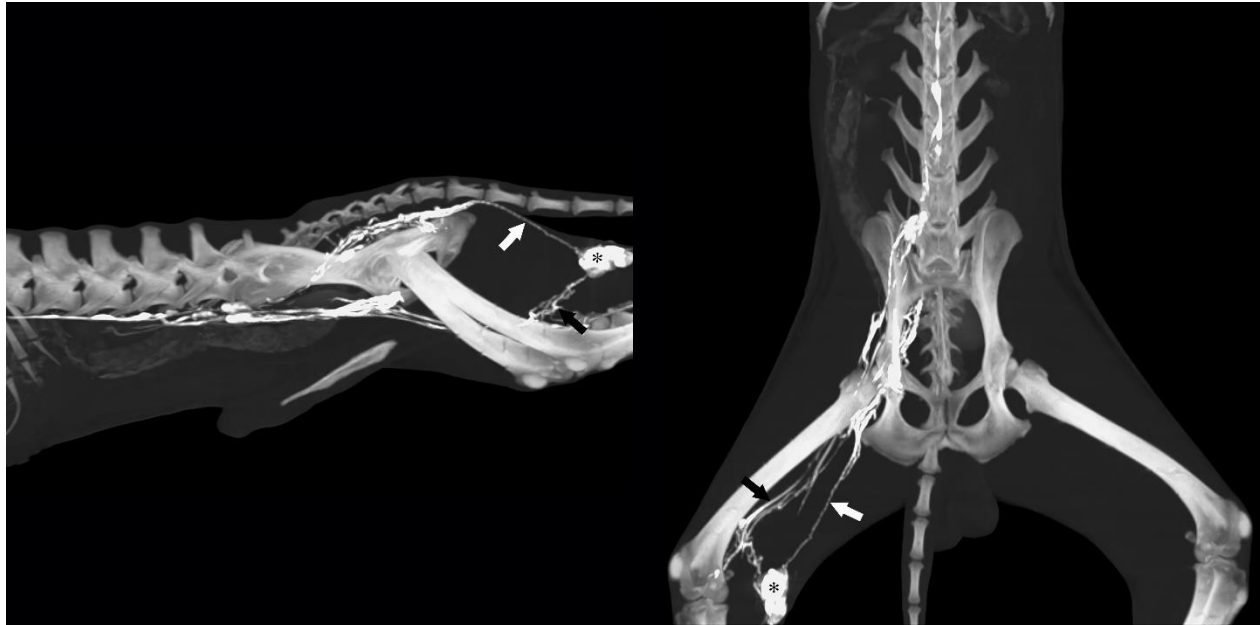


Figure 2.8.4. Left lateral and dorsoventral maximum intensity projections of a dog 5 minutes after percutaneous injection of 10 ml of 350 mgI/ml iohexol into the left popliteal lymph node. In addition to direct drainage from the popliteal lymph node (*) to the medial iliac lymph node (efferent vessels indicated by black arrows), this dog has a second pathway in which efferent lymphatics course laterally along the hind limb, then pass dorsally to the pelvic bones and drain to nodes caudal to the medial iliac lymph nodes (efferent vessels indicated by white arrows).

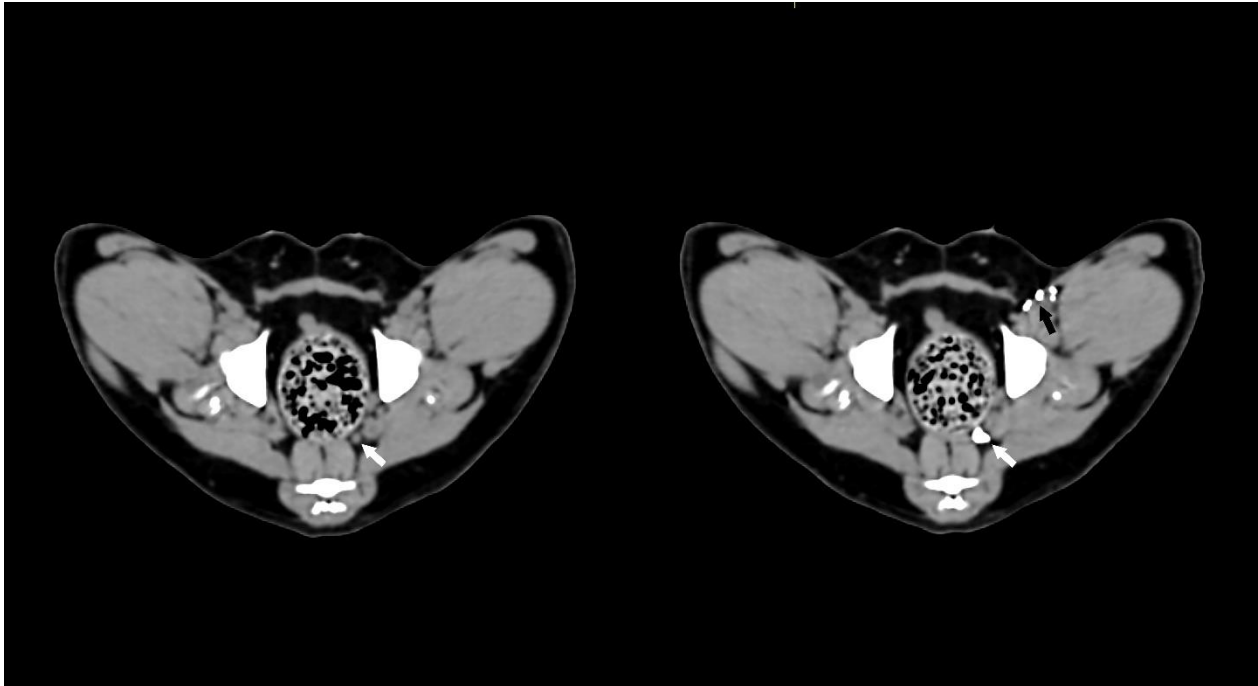


Figure 2.8.5. Transverse computed tomographic image of a dog pre- (left) and 5 minutes post-injection of 4.2 ml of 350 mgI/ml iohexol (right) into the left popliteal lymph node. The white arrows indicate a lymph node adjacent to the rectum that was entered by lymphatic vessels coursing over top of the pelvic bones. The black arrow indicates lymphatic vessels passing from the popliteal lymph node directly to the medial iliac lymph node.

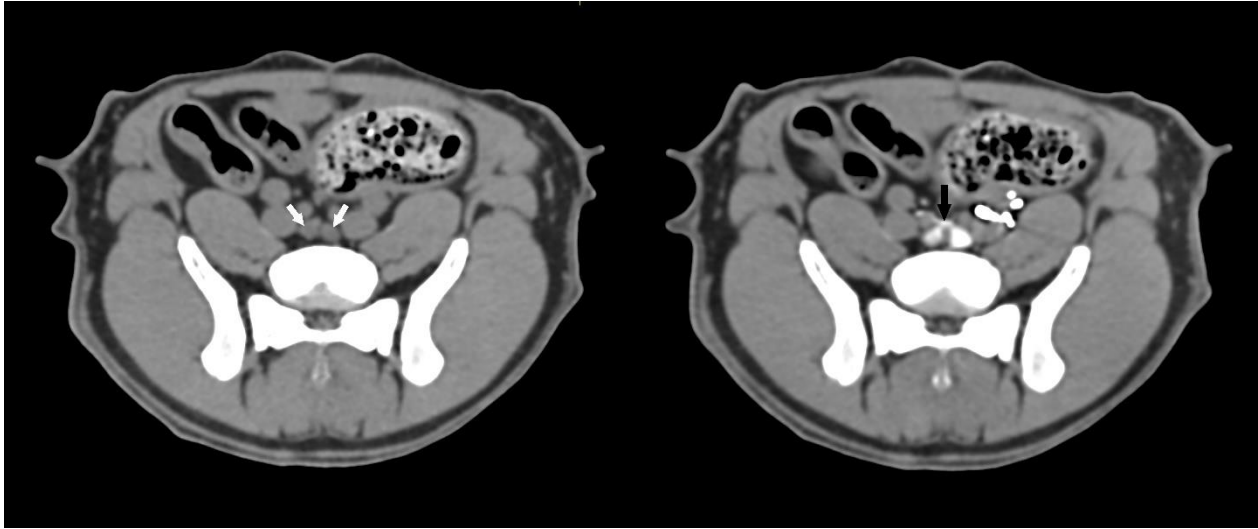


Figure 2.8.6. Transverse computed tomographic image of a dog pre- (left) and 5 minutes post-injection of 10 ml of 350 mgI/ml iohexol (right) into the left popliteal lymph node. The white arrows indicate the right and left internal iliac lymph nodes, and the black arrow indicates contrast in a vessel connecting the two nodes. The efferent lymphatic vessels coursing from the popliteal lymph node to the medial iliac lymph nodes are visible to the right of the black arrow.

2.9 Transition to Chapter 3

During the course of the computed tomography lymphography study, contrast flow from the popliteal lymph node to the ipsilateral or ipsilateral and contralateral medial iliac lymph nodes was observed in all dogs as previously described. Radiation therapy for known or suspected disease in the medial iliac lymph nodes is common practice when treating a solid tumour in any body area drained by these lymph nodes. Although it is becoming less common, treatment without three-dimensional imaging is still performed, so the location of the medial iliac lymph nodes with respect to anatomy that is palpable or identifiable on two-dimensional port imaging is required. A literature review revealed only sparse information on treatment of these lymph nodes with manual setup or port film imaging only, and treatment recommendations varied among these few papers. This led to the study describing the location of normal-sized medial iliac lymph nodes with respect to bony anatomy.

3. Location of Normal-Sized Medial Iliac Lymph Nodes Relative to Lumbar Vertebrae in 100 Dogs

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At the time of preparation of this thesis, this manuscript has not been submitted for publication.

My contributions to this study included formulation of the objective, study design, coordinating with staff and faculty at Colorado State University Veterinary Teaching Hospital to enable data collection from their patients, data collection with Dr. Yoshikawa, assistance in data interpretation and analysis, and assistance in literature review and manuscript writing and editing.

3.1 Abstract

Irradiation of normal-sized medial iliac lymph nodes may be performed when malignant cells are detected within the nodes, or when the nodes are suspected to contain subclinical disease. When three-dimensional imaging has not been performed, radiation field margins are based on the presumed location of the nodes relative to the lumbar vertebrae. The objective of this study was to describe the location of the medial iliac lymph nodes relative to lumbar vertebral bodies. Computed tomographic scans of 100 dogs with no known disease of the medial iliac lymph nodes were reviewed. The nodes were contoured using radiation treatment planning software, and the position of the nodes relative to the vertebral bodies was measured using sagittal and dorsal plane digitally reconstructed radiographs. There was no difference in cranial or caudal extent of the nodes between dogs in dorsal and lateral recumbency, however, there was a significant difference in the distance of the ventral margin of the nodes from the vertebral bodies between dogs in dorsal and dogs in lateral recumbency ($p < 0.001$). Based on this population of dogs, radiation treatment fields for normal-sized medial iliac lymph nodes should encompass the fifth through seventh vertebral body in the cranial to caudal direction, for dogs in both dorsal and lateral recumbency. The treatment fields should extend two times the height of the sixth lumbar vertebra in the dorsal to ventral direction for dogs in dorsal recumbency, and two-and-a half times the height of the sixth lumbar vertebra for dogs in lateral recumbency.

3.2 Introduction

Irradiation of normal-sized medial iliac lymph nodes may be performed when malignant cells are detected within the nodes, or when the nodes are suspected to contain subclinical disease for cancers such as mast cell tumors, genitourinary carcinomas and anal sac adenocarcinoma.^{1,2} With cross-sectional imaging, a radiation oncologist can accurately delineate the medial iliac lymph nodes for the purpose of treatment planning, however, cross-sectional imaging is not always used for treatment planning. A radiation oncologist may elect a simpler beam arrangement for regions of relatively uniform thickness, such as the lumbar region, as a predictable dose distribution can be achieved with less planning time, a simpler patient setup and less expense. When cross-sectional imaging is not available, the location of the medial iliac lymph nodes must be estimated based on landmarks that can be palpated or are visible on portal imaging, as normal-sized nodes are not visible on kilovoltage or megavoltage portal radiographs.³ The location of normal-sized medial iliac lymph nodes relative to lumbar vertebrae has been described in a limited number of dogs.⁴⁻⁶ The nodes, usually single but at times double on one or both sides, have been described as lying ventral to the bodies of the fifth and sixth lumbar vertebrae.⁴ Investigations using lymphangiography have suggested that the nodes are located more caudally, ventral to the bodies of the sixth and seventh lumbar vertebrae.^{5,7} Computed tomographic (CT) examination of 42 presumed normal medial iliac lymph nodes in 19 dogs revealed that all nodes were located ventral to the sixth and/or seventh lumbar vertebrae, with the exception of two nodes that extended cranially to lay under the fifth lumbar vertebra.

Parallel-opposed fields from the right and left sides are commonly used to treat the lumbar region, necessitating a decision by the radiation oncologist as to field margins not only in the cranial-caudal direction, but also in the dorsal-ventral direction.^{1,2} Due to the proximity of the colon, it is optimal to limit the ventral extent of the radiation field to no more than needed to target the lymph nodes, however, to the authors' knowledge there is no information available on the extent of normal-sized nodes ventral to the vertebrae. The objective of this study was to describe the location of normal-sized medial iliac lymph nodes relative to lumbar vertebral bodies in the three basic anatomical planes.

3.3 Materials and Methods

This study protocol was submitted to the University of Saskatchewan's Animal Research Ethics Board and was determined to be exempt from review.

Computed tomographic scans of 100 dogs were identified in the picture archiving and communication system (PACS) of the Western College of Veterinary Medicine Veterinary Medical Centre and the Colorado State University Veterinary Teaching Hospital that met the following inclusion criteria: (1) included the medial iliac lymph nodes, (2) slice thickness less than or equal to 2 millimeters, (3) dogs positioned in lateral recumbency ($n = 50$) or dorsal recumbency ($n = 50$), and (4) node sizes that did not exceed described normal ranges. Previously described size and length-to-width ratio limits for 200 dogs with presumed normal medial iliac lymph nodes were used to determine exclusion criteria. The length, width, height and ratio of length to width of the largest medial iliac lymph node on the left and right side of each dog was measured using the previously described method. Dogs were excluded if they met one or more of the following criteria: (1) if the length of a medial iliac lymph node was $> 3.1\text{cm}$ (dogs $\leq 15\text{kg}$) or $> 4.5\text{cm}$ (dogs $> 15\text{kg}$), (2) if the width of a medial iliac lymph node was $> 0.9\text{cm}$ (dogs $\leq 15\text{kg}$) or $> 1.5\text{cm}$ (dogs $> 15\text{kg}$), (3) if the height of a medial iliac lymph node was $> 0.5\text{cm}$ (dogs $\leq 15\text{kg}$) or $> 0.9\text{cm}$ (dogs $> 15\text{kg}$), and (4) if the length to width ratio of a medial iliac lymph node was < 1.5 (dogs $\leq 15\text{kg}$). A length to width ratio of < 2.0 was applied to dogs $> 15\text{kg}$ based on other published values.^{3,8} The publication used for the exclusion criteria did not suggest a length-to-width ratio limit for dogs $> 15\text{kg}$.

Scans at Colorado State University were acquired using a 16-slice PET-CT scanner (Gemini TF Big Bore System, Philips Medical System, Cleveland, OH), and scans at the Western College of Veterinary Medicine were acquired using a 16-slice CT scanner (Acquilion 16, Toshiba America Medical Systems, Tustin, CA). Age, weight, gender, neuter status, breed and reason for CT were recorded for each dog.

The medial iliac lymph nodes were manually contoured using a drawing tool in a radiation treatment planning system (Eclipse v10, Varian Medical Systems Inc., Palo Alto, CA), with author consensus (KAS, MNM, HY) on the contour definition. Digitally reconstructed radiographs were generated in the sagittal and frontal planes, and the position of the lymph nodes was measured relative to the lumbar vertebral bodies. The cranial and caudal extent of the nodes

was measured relative to the overlying vertebral bodies, the dorsal and ventral extent was measured relative to the height of the cranial end of the sixth lumbar vertebral body, and the right and left extent was measured relative to the width of the cranial end of the sixth lumbar vertebral body (Figure 1). Cranial caudal location was coded as follows: 1 to 6 for cranial half of L5 to caudal half of L7. Dorsal ventral location was coded 1 to 6 for 0.0-0.5 to 2.5-3.0 times the height of the sixth lumbar vertebra. The right and left lateral extent was measured as either 1 or 2 widths of the sixth lumbar vertebra.

Differences between medial iliac lymph node extent for dogs in dorsal and lateral recumbency were examined using multivariable Box-Cox regression adjusting for study site, age, weight, and sex. Box-Cox regression was chosen as the coded location of the lymph nodes relative to the vertebral bodies were not normally distributed, routine transformation did not achieve normality, and multivariable analysis was required. Statistical tests were selected and completed by an analytic epidemiologist using a commercial statistical software (STATA/SE version 14.1 for Windows, StataCorp, College Station, TX). P-values ≤ 0.05 were considered statistically significant.

The location of the nodes relative to the dorsal margin of the colon was also examined. The colon was manually contoured at the level of the medial iliac lymph nodes, and the presence or absence of overlap of the contours of the colon and the medial iliac lymph nodes was evaluated on a digitally reconstructed radiograph generated in the sagittal plane (Figure 2). The overlap of the nodes and the colon was categorized as $< 25\%$, $\geq 25\%$ to $< 50\%$, $\geq 50\%$ to $< 75\%$, and $\geq 75\%$ to 100% of the dorsal to ventral height of the colon.

3.4 Results

Dogs underwent CT scanning for diagnostic purposes for a presumptive diagnosis of intervertebral disc herniation (n = 23), orthopedic abnormality (n = 4), portosystemic shunt (n = 2), spinal cord disease (n = 2), for follow-up of a nephroblastoma that had been surgically excised (n = 1), and for radiation treatment planning purposes for genitourinary neoplasia (n = 29), soft tissue sarcoma (n = 12), primary bone neoplasia (n = 7), thyroid neoplasia (n = 1), infiltrative lipoma (n = 1), oral tumor (n = 1), and apocrine gland adenocarcinoma (n = 1). Sixteen clinically normal dogs were imaged for research purposes. Computed tomographic scans acquired between January 2011 and July 2016 were included in the study. Slice thickness included 0.8mm (n = 7 dogs), 1.0mm (n = 29 dogs) and 2.0mm (n = 64 dogs). Of the 50 dogs in lateral recumbency, 34 were in right lateral recumbency and 16 were in left lateral recumbency.

The median age of the dogs was 8.5 years (range, 8 months to 15 years). The median body weight was 16.8kg (range, 2.1 – 73.0kg). There were 48 neutered males, 36 neutered females, 10 intact females and 6 intact males. Breeds included mixed (n = 37), Dachshund (n = 12), Beagle (n = 5), Labrador Retriever (n = 4), Border Collie (n = 3), German Shepherd (n = 3), Bernese Mountain Dog (n = 2), Chihuahua (n = 2), Collie (n = 2), German Shorthaired Pointer (n = 2), Golden Retriever (n = 2), Maltese (n = 2), Miniature Schnauzer (n = 2), Scottish Terrier (n = 2), Shetland Sheep Dog (n = 2), Shih Tzu (n = 2), and 1 each of Australian Shepherd, Bedlington Terrier, Bichon Frise, Cocker Spaniel, Fox Terrier, German Wirehaired Pointer, Greyhound, Husky, Miniature Poodle, Newfoundland, Pekingese, Schipperke, Standard Poodle, Weimeraner, West Highland White Terrier, and Whippet.

The medial iliac lymph nodes were single on both sides in 57 dogs, doubled on the left side in 31 dogs, doubled on the right side in 6 dogs, and doubled on both sides in 5 dogs. One dog had 2 nodes on the left side and 3 nodes on the right side.

The cranial and caudal extent of the medial iliac lymph nodes relative to the lumbar vertebrae are presented in Table 1. There was no difference between dogs in dorsal recumbency and dogs in lateral recumbency for cranial extent of the lymph nodes ($p = 0.98$) or caudal extent of the lymph nodes ($p = 0.30$).

The position of the medial iliac lymph nodes relative to the lumbar vertebral bodies in the dorsal to ventral direction is presented in Table 2. There was no difference in the distance of the

dorsal margin of the lymph nodes from the vertebral bodies between dogs in dorsal recumbency and dogs in lateral recumbency ($p = 0.41$). However, there was a significant difference in the distance of the ventral margin of the lymph nodes from the vertebral bodies between dogs in dorsal recumbency and dogs in lateral recumbency ($p < 0.001$). The ventral margin of the lymph nodes was located at a greater distance from the ventral border of the vertebral bodies for dogs in lateral recumbency than for dogs in dorsal recumbency. For dogs in dorsal recumbency, the distance of the ventral margin of the nodes from the ventral border of the vertebral bodies was between 1.5-2 times the height of the sixth lumbar vertebra in 8% (4/50) of dogs, and between 2-2.5 times the height of the sixth lumbar vertebra in none of the dogs. In contrast, for dogs in lateral recumbency, the distance of the ventral margin of the nodes from the ventral border of the vertebral bodies was between 1.5-2 times the height of the sixth lumbar vertebra in 32% (16/50) of dogs and between 2-2.5 times the height of the sixth lumbar vertebra in 6% (3/50) of dogs.

In 98% (98/100) of dogs, the left and right lateral extent of the nodes were within 1 width of the sixth lumbar vertebra from the lateral margin of the vertebral body. In 2% (2/100) of dogs, the right lateral extent of the nodes was within 2 sixth lumbar vertebra widths from the lateral margin of the vertebral body, while the left lateral extent of the nodes was within 1 sixth lumbar vertebra width. There was no difference between dogs in dorsal recumbency and dogs in lateral recumbency for the right lateral extent of the nodes ($p = 0.68$).

There was overlap of the medial iliac lymph nodes and the colon in the sagittal plane in 48% (48/100) of dogs. The overlap of the nodes and the colon was categorized as $< 25\%$ in 43% (43/100) of dogs, as $\geq 25\%$ to $< 50\%$ in 3% (3/100) of dogs, and as $\geq 50\%$ to $< 75\%$ in 2% (2/100) of dogs. Overlap of nodes and colon occurred ventral to the fifth, sixth and seventh lumbar vertebrae.

3.5 Discussion

Dorsal and lateral recumbency were selected as these are the two most common positions in which dogs would be irradiated when two-dimensional treatment planning is used. To meet the inclusion criterion of lateral positioning, it was necessary to use dogs scanned for radiation treatment planning purposes. Other dogs scanned in lateral position at the two sites were mostly trauma patients, and there was a low number of these dogs. The use of radiation planning CT scans did lead to the inclusion of dogs that had been diagnosed with a malignant neoplasm of the caudal half of the body. While none of these dogs had been diagnosed with metastasis to the medial iliac lymph nodes, it is possible that the lymph nodes contained microscopic metastases. However, all the nodes fell within the previously reported size ranges for presumed normal medial iliac lymph nodes, and therefore we feel that the results of this study can be applied to normal-sized nodes.

Cross-sectional imaging-based treatment planning is now commonly used in veterinary oncology. With this method of planning, segmentation of targeted lymph nodes is used to ensure dose coverage. However, for certain clinical scenarios, such as irradiation of a cutaneous tumor on a hind limb, cross-sectional imaging such as CT may not improve the probability of tumor control or decrease the risk of adverse effects, and the additional cost of a CT scan and increased planning time may not be warranted. In these cases, if normal-sized medial iliac lymph nodes have been confirmed to contain metastatic disease, or if prophylactic irradiation is prescribed, the radiation oncologist must define a treatment field that will encompass the nodes. The normal-sized medial iliac lymph nodes are not visible on kilovoltage or megavoltage portal imaging, so the lumbar vertebrae, which are visible, are commonly used as landmarks to estimate the position of the lymph nodes. Radiation beams entering from the lateral aspect of the patient are commonly used, and therefore the extent of the nodes relative to the lumbar vertebrae in the cranial to caudal and dorsal to ventral directions is most useful when defining the radiation field margins.^{1,2} The right to left extent is less important given the shape of the dose distribution when right and left parallel opposed beams are used.

To the authors' knowledge, only one publication describes the radiation field margins used for two-dimensional treatment planning for normal-sized medial iliac lymph nodes.¹ In a retrospective study of 19 dogs treated for cutaneous mast cell tumors, the medial iliac and

hypogastric lymph nodes were irradiated prophylactically in 11 dogs. The radiation field extended from the caudal end of L5 to the cranial end of S1. In another study that included dogs diagnosed with anal sac adenocarcinoma in which normal-sized medial iliac lymph nodes were irradiated, the cranial extent of the radiation field for one dog appears to be at the caudal aspect of the fifth lumbar vertebra, based on a portal film.² Based on our results, these fields would not have included the cranial extent of at least one medial iliac node in 45% of dogs.

Position of the dogs did not impact the node position, with the exception of the nodes extending more ventrally when the dogs were in lateral recumbency than in dorsal recumbency. This is likely due to the displacement of the nodes towards the spine caused by the weight of the abdominal organs when the dogs were laying on their backs.

When manually planning medial iliac lymph node fields, radiation oncologists could choose to limit the ventral extent of the treated volume to the dorsal margin of the colon, to decrease dose to the colon. For this reason we examined overlap between the medial iliac lymph nodes and the colon, and found that limiting the treated volume by the colon would lead to a portion of the nodes not being included in the treated volume in about half the dogs. The position of the colon varies day-to-day, and the estimation of overlap percentage was made to give a rough idea of the magnitude of overlap, not to guide field setup.

In conclusion, based on this population of dogs, radiation treatment fields for normal-sized medial iliac lymph nodes should encompass the fifth through seventh vertebral body in the cranial to caudal direction, for dogs in both dorsal and lateral recumbency. In the dorsal to ventral direction, the treatment fields should extend two times the height of the sixth lumbar vertebra for dogs in dorsal recumbency, and two-and-a half times the height of the sixth lumbar vertebra for dogs in lateral recumbency.

3.6 References

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3.7 Tables

Table 3.7.1. Cranial and Caudal Extent of Normal-Sized Medial Iliac Lymph Nodes Relative to the Lumbar Vertebrae in 100 Dogs				
Cranial Margin	5TH LUMBAR VERTEBRA		6TH LUMBAR VERTEBRA	
	Cranial Half	Caudal Half	Cranial Half	Caudal Half
	7% (7/100)	38% (38/100)	37% (37/100)	18% (18/100)
Caudal Margin	6TH LUMBAR VERTEBRA		7TH LUMBAR VERTEBRA	
	Cranial Half	Caudal Half	Cranial Half	Caudal Half
	1% (1/100)	31% (31/100)	43% (43/100)	25% (25/100)

Table 3.7.2. Distance of the Dorsal and Ventral Margins of Normal-Sized Medial Iliac Lymph Nodes from the Ventral Border of the Vertebral Bodies in 100 Dogs, with Distance Described Relative to the Cranial End of the Sixth Lumbar Vertebra

FRACTION OF L6^a HEIGHT	DORSAL MARGIN	VENTRAL MARGIN
0-0.5	71% (71/100)	0
0.5-1.0	25% (25/100)	29% (29/100)
1.0-1.5	4% (4/100)	48% (48/100)
1.5-2.0	0	20% (20/100)
2.0-2.5	0	3% (3/100)

^aSixth Lumbar Vertebra

3.8 Figures

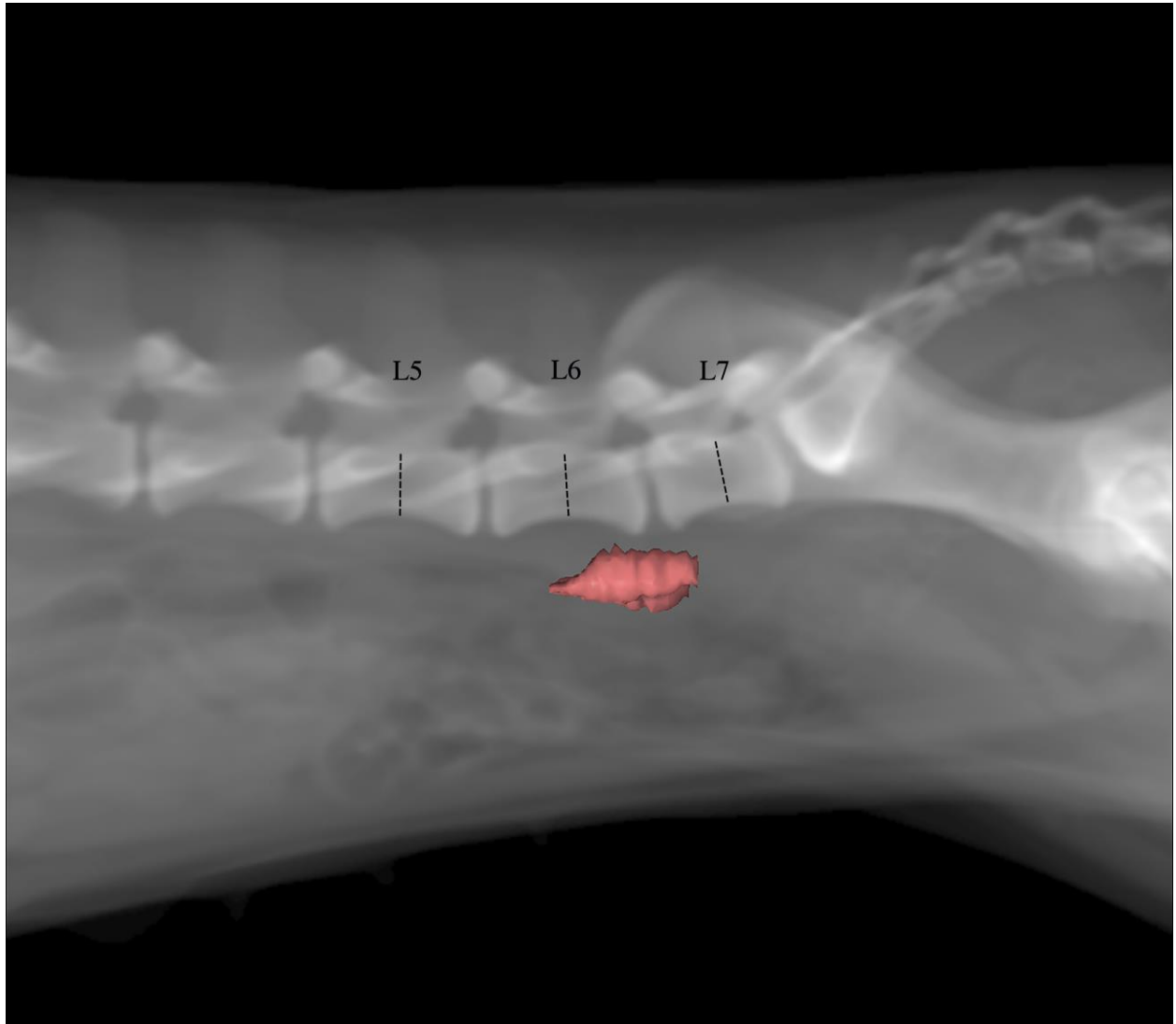


Figure 3.8.1A. A sagittal plane digitally reconstructed radiograph of a dog with normal-sized medial iliac lymph nodes that have been manually contoured. The cranial and caudal extent of the medial iliac lymph nodes were measured relative to the cranial or caudal half of the overlying vertebral bodies. The lymph nodes in this dog extend from the cranial half of the sixth lumbar vertebra (L6) to the cranial half of the seventh lumbar vertebra (L7).

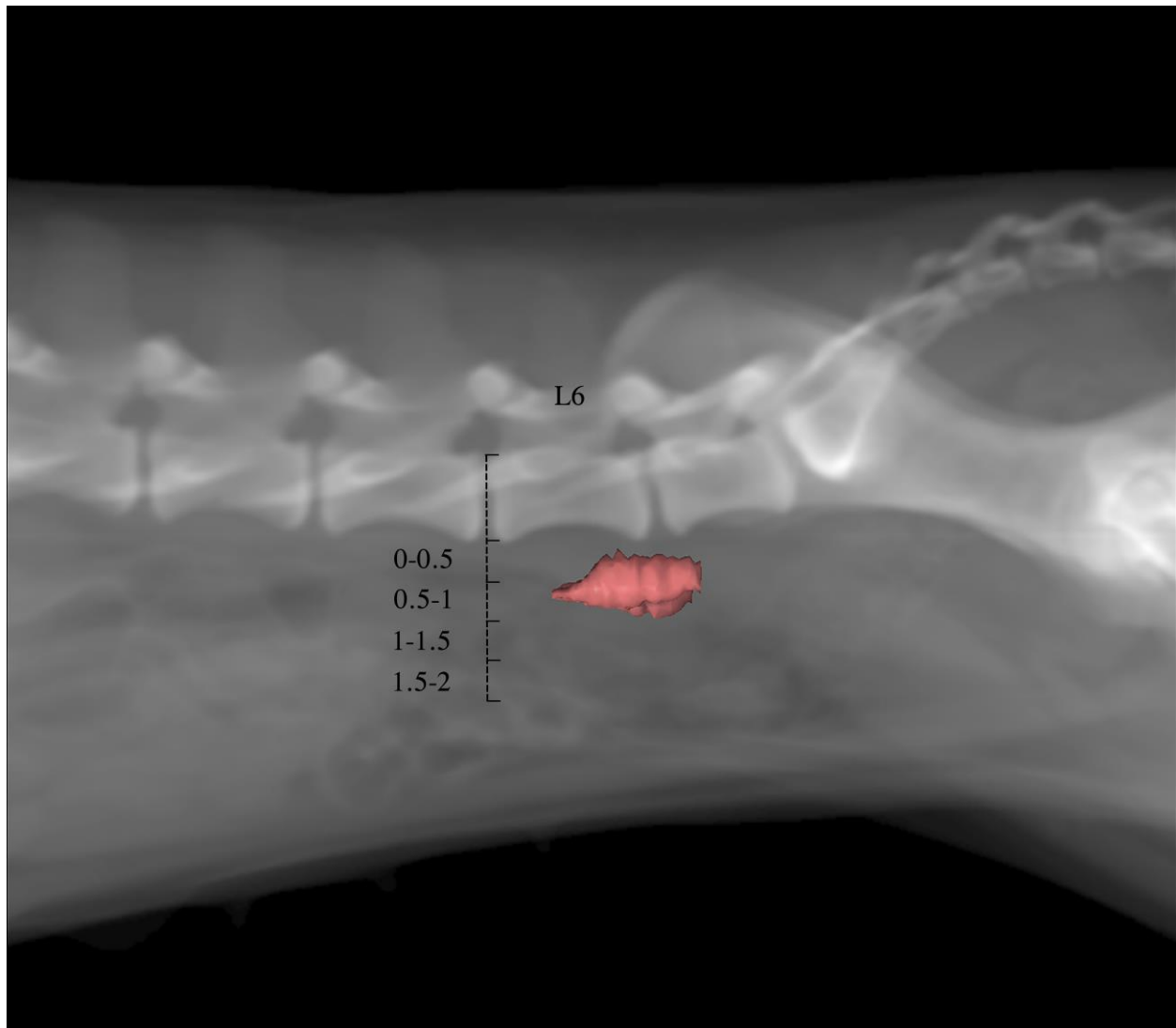


Figure 3.8.1B. The dorsal and ventral extent of the medial iliac lymph nodes were measured relative to the height of the cranial end of the sixth vertebral body (L6). The dorsal extent of the lymph nodes in this dog is within 0-0.5 times the height of L6, and the ventral extent is within 0.5-1 times the height of L6.

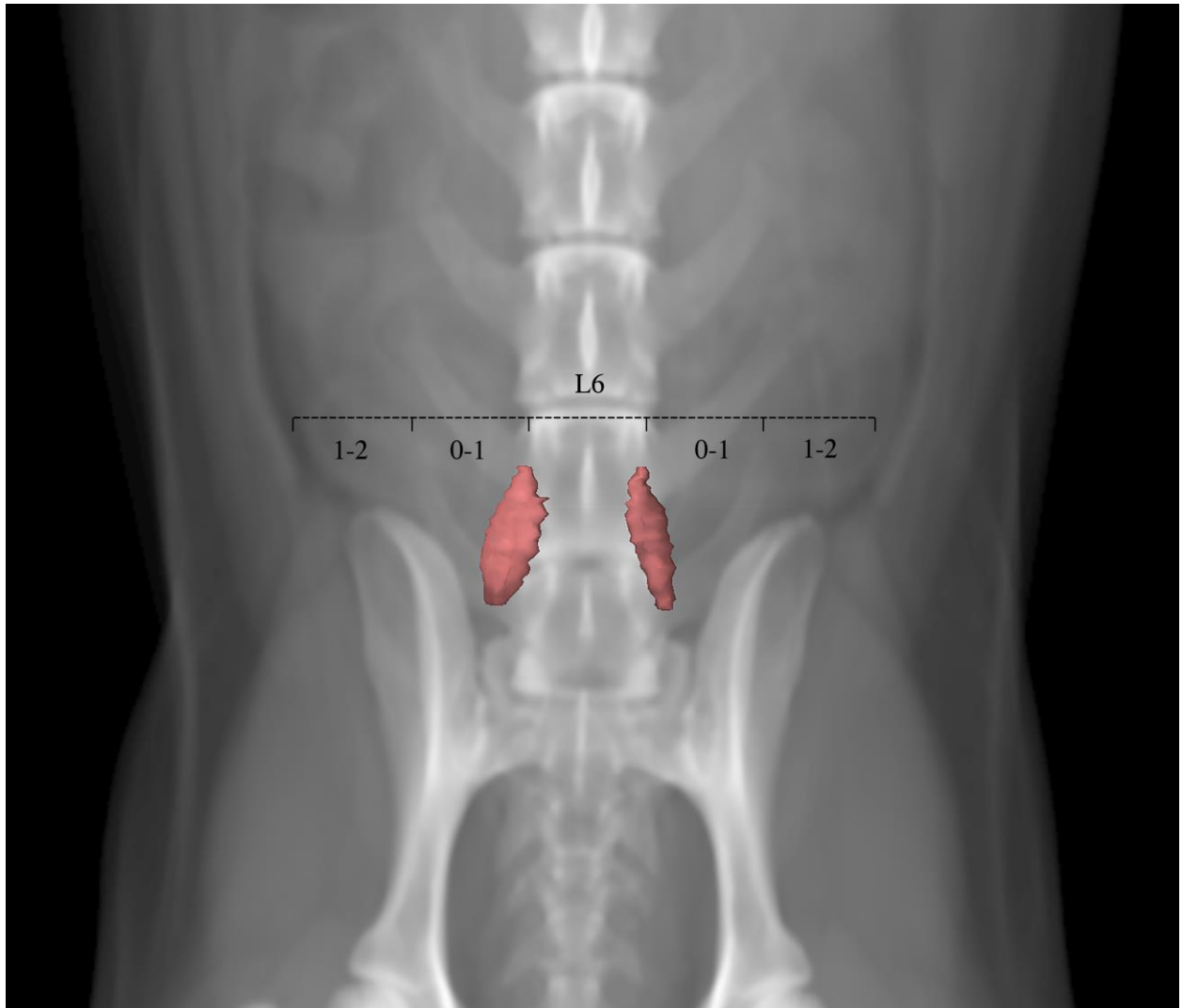


Figure 3.8.1C. A dorsal plane digitally reconstructed radiograph of the same dog as in Figure 1A and 1B. The right and left most lateral extent of the medial iliac lymph nodes were measured relative to the width of the cranial end of the sixth vertebral body (L6). The left and right most lateral extent of the nodes in this dog are within 1 times the width of L6.

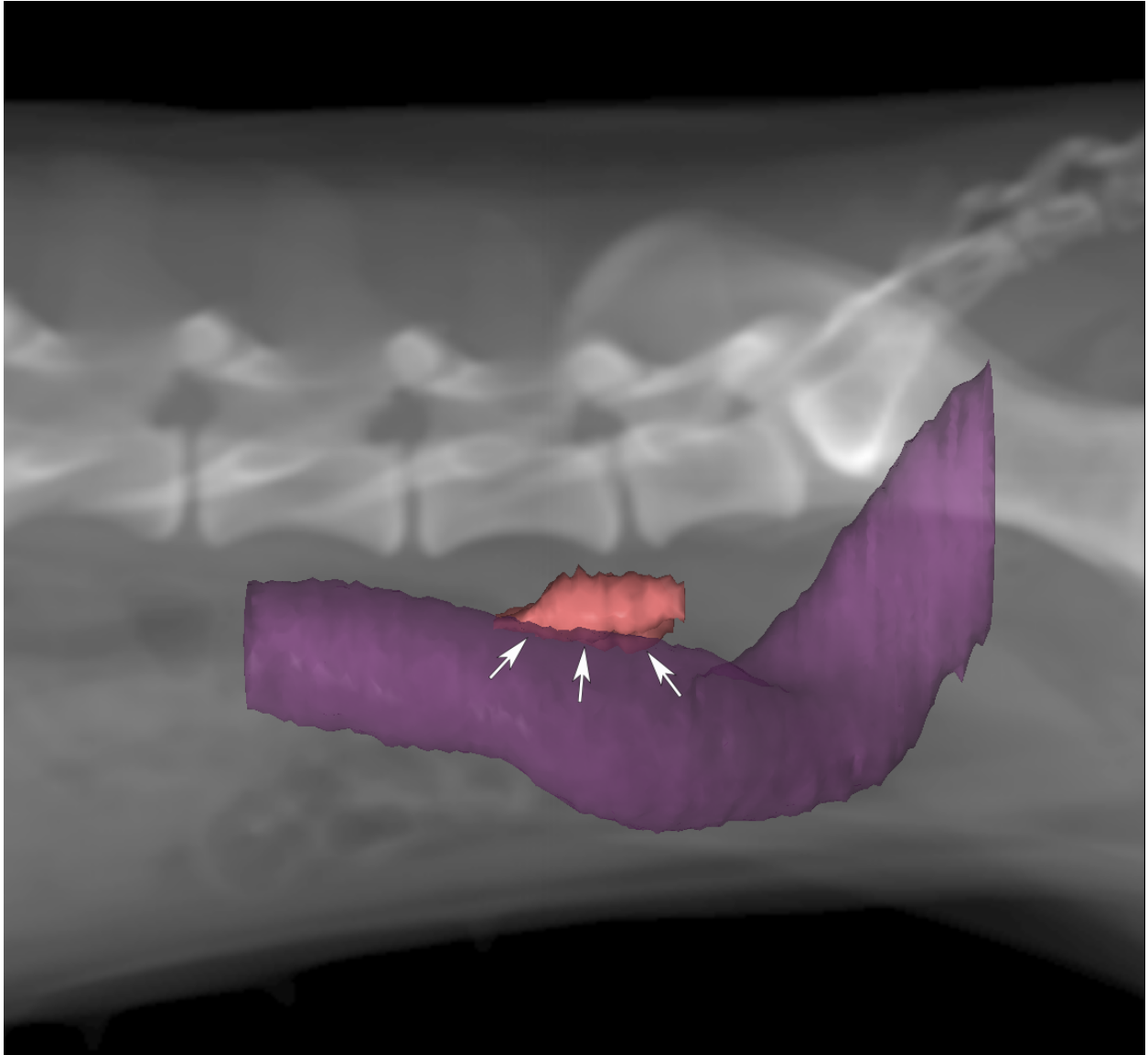


Figure 3.8.2. A digitally reconstructed radiograph generated in the sagittal plane showing the position of the colon relative to the medial iliac lymph nodes. The presence or absence of overlap of the two contours was noted. In this dog, there was overlap present (white arrows), categorized as less than 25% of the dorsal to ventral height of the colon.

3.9 Transition to Chapter 4

Irradiation of normal-sized medial iliac lymph nodes may be performed when malignant cells are detected or suspected. In order to choose appropriate inclusion criteria for the location of normal-sized medial iliac lymph nodes, a literature review was performed to find a reference range for the size of canine medial iliac lymph nodes. One published study on computed tomographic characteristics of the abdominal lymph nodes reported the range and median dimensions of only 42 lymph nodes measured in 19 dogs. Another paper reported the mean dimensions of normal and abnormal medial iliac lymph nodes in twelve dogs, but all of these patients had been diagnosed with anal sac gland adenocarcinoma. Neither of these available studies included sufficient patient numbers to determine a reference range, and one included dogs with known neoplastic disease in an area that drains to the nodes in question. This led to the next study on computed tomographic characteristics of presumed normal canine medial iliac lymph nodes. In order to develop a de novo reference range, computed tomographic scans of 200 dogs without suspicion of disease in the lymph nodes were included.

4. Characteristics of Presumed Normal Canine Medial Iliac Lymph Nodes on Computed Tomographic Examination

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At the time of preparation of this thesis, this manuscript has not been submitted for publication.

My contributions to this study included formulation of the objective, study design, coordinating with staff and faculty at Colorado State University Veterinary Teaching Hospital to enable data collection from their patients, data collection, assistance in data interpretation and Analysis, and assistance in literature review and manuscript writing and editing.

4.1 Abstract

Nodal size is the most widely accepted criterion to discriminate between normal and pathologic lymph nodes on computed tomographic (CT) examination. The size of normal medial iliac lymph nodes on CT imaging has been described in a very limited number of dogs. The objective of this study was to develop reference intervals for size and x-ray attenuation for canine medial iliac lymph nodes on CT examination, using scans of 200 dogs with no known disease of the medial iliac lymph nodes. The length, width, height and x-ray attenuation of the largest medial iliac lymph node on the left and right side of each dog were measured. Reference intervals were developed following the American Society for Veterinary Clinical Pathology guidelines. Potential factors explored for including as a partitioning factor for calculated reference intervals included site scan was performed, side of body (left or right), age, weight, gender, neuter status, and breed. Reference intervals were calculated from 95% percentiles of lymph node characteristics where > 120 samples were available. Where > 40 and < 120 samples were available, robust estimators were used. Weight was significantly associated with width, length, and height measurements of lymph nodes ($P < 0.0001$) and was retained as a partitioning factor. For dogs ≤ 15 kg, suggested limits for defining normal medial iliac lymph nodes are: width ≤ 0.9 cm, height ≤ 0.5 cm, length ≤ 3.1 cm and length-to-width ratio ≥ 1.5 . For dogs > 15 kg, suggested limits are: width ≤ 1.5 cm, height ≤ 0.9 cm, and length ≤ 4.5 cm.

4.2 Introduction

Lymph flowing through a malignant tumor located in the caudal body wall will drain to the medial iliac lymph nodes (MILNs), which are the largest and most constant nodes of the iliosacral lymph center in the dog.¹ These nodes receive efferent vessels from parietal structures of the abdominal and pelvic walls, including skin in the abdominal, pelvic and thigh regions, the muscles of the hind limb and pelvis, the male and female genital organs, and the anus and rectum.¹ They also receive efferent vessels from the other iliosacral lymph center nodes, the inguinal lymph nodes and the popliteal lymph nodes.¹ The presence of metastatic disease in the MILNs in dogs with malignant neoplasia affects prognosis and patient management for many tumor types.² Canine MILNs are consistently visualized on computed tomographic (CT) examination, and CT is used for discriminating between malignant and benign nodes, using features such as size, shape, and nodal morphology.^{3,4} To define abnormal canine MILNs on CT examination, radiologic features of normal MILNs are needed.

Nodal size is the most widely accepted criterion to discriminate between normal and pathologic lymph nodes on CT examination.⁴ Because lymph nodes are likely to become rounder before elongating, the maximum short-axis diameter has been recommended to measure nodal size in human patients.⁴ The size of normal MILNs on computed tomographic imaging has been described in a very limited number of dogs.^{3,5,6} In a study of CT characteristics of abdominal lymph nodes in 19 adult dogs with no disease process that might be expected to affect the size of the MILNs, the transverse and longitudinal diameters of 42 medial iliac lymph nodes was described.³ The mean length (maximal dimension), width (second maximal dimension) and thickness (dimension perpendicular to width) were 22.8mm (range, 3.0-56.0mm), 6.7mm (2.4-11.2mm) and 4.6mm (1.2-9.6mm). Another study reported transverse and longitudinal dimensions of 29 normal MILNs in 12 dogs examined with CT.⁵ However, all the dogs in this study had anal sac gland carcinoma, and lymph nodes were subjectively deemed normal or abnormal based on imaging characteristics only. A 2016 study described MILN size on CT in 30 adult dogs in which underlying disease was determined not to affect the iliosacral lymphcenter.⁶ The mean maximum transverse diameter of the largest MILN was 7.4mm +/- 2.8mm.

Nodal shape has also been suggested to discriminate between malignant and benign lymph nodes, however, there is limited information on the shape of normal MILNs in dogs.⁴ The study of 19 adult dogs with 42 presumed normal MILNs classified nodal shape based on the ratio

of width to length, with a node that had a width equal to or less than half the length reported as elongated, and a node with a width greater than half the length reported as rounded.³ This study found that 76% (32/42) of MILNs were elongated, 7% (3/42) were rounded, and 17% (7/42) could not be clearly placed in either category. Other authors have described a longitudinal-to-transverse diameter ratio of equal to, or greater than, 2:1 for normal lymph nodes.^{7,8} In addition to shape, nodal x-ray attenuation has been used to determine metastatic involvement of lymph nodes in human patients.⁴

The objective of this study was to develop reference intervals for lymph node size, length-to-width ratio, and x-ray attenuation for canine medial iliac lymph nodes on CT examination, using CT scans of 200 dogs with no known disease of the medial iliac lymph nodes.

4.3 Materials and Methods

This study protocol was submitted to the University of Saskatchewan's Animal Research Ethics Board and was determined to be exempt from review. A search of the picture archiving and communication system (PACS) of the Western College of Veterinary Medicine Veterinary Medical Centre and the Colorado State University Veterinary Teaching Hospital was performed for computed tomographic (CT) scans of dogs that included the medial iliac lymph nodes (MILNs), and had a slice thickness less than or equal to 2 millimeters. The medical records for the dogs identified in this manner were reviewed, and dogs were excluded if they met one or more of the following criteria: (1) if the history or diagnosis included injury, infection, or disease of a region that drains to the MILNs, (2) if the bladder was in direct contact with a MILN, (3) if there was a diagnosis (presumptive or confirmed) of lymphoma, mast cell tumor, or leukemia, and (4) mention of MILN lymphadenopathy, peritonitis, or peritoneal effusion in the radiologist interpretation. The search was started at the current date and continued until 100 CT scans from each institution were identified, resulting in inclusion of CT scans from November 2014 to July 2016 for the Western College of Veterinary Medicine Veterinary Medical Centre, and from August 2013 to September 2016 for the Colorado State University Veterinary Teaching Hospital. Age, weight, gender, neuter status and breed were recorded for each dog.

Scans at Colorado State University were acquired using a 16-slice PET-CT scanner (Gemini TF Big Bore System, Philips Medical System, Cleveland, OH), and scans at the Western College of Veterinary Medicine were acquired using a 16-slice CT scanner (Acquilion 16, Toshiba America Medical Systems, Tustin, CA). The following scan parameters were used at Colorado State University: 120 kVp, 69-469 mA, tube rotation time 0.5 s, and 512 x 512 matrix dimensions. At the Western College of Veterinary Medicine, scan parameters were as follows: 100-135 kVp, 200-300 mA, tube rotation time 0.5 s, and 512 x 512 matrix dimensions.

The measurements recorded in millimeters for each dog included the length, width, and height of the largest MILN on the left and right side of each dog. All lymph node measurements were made by a single investigator, using soft tissue settings. A sagittal reconstruction was used to subjectively determine the thickest portion of each MILN. The axial slice corresponding with that location was used to measure the greatest diameter of the lymph node, and this was recorded as width. Height was defined as the diameter perpendicular to the width measurement. Lastly, the length was obtained by multiplying the slice thickness by the number of axial slices on which

the lymph node was visible. The ratio of length to width was calculated for each node. If more than one MILN was present on the right or left side, the largest lymph node on that side was defined as the node with the greatest width. X-ray attenuation in Hounsfield units (HU) was measured using the PACS elliptical region-of-interest tool, using a region of interest made as large as possible on the axial slice used for width and height measurement.

Development of reference intervals followed American Society for Veterinary Clinical Pathology guidelines.⁹ Potential factors explored for including as a partitioning factor for calculated reference intervals for lymph node size and x-ray attenuation included site CT was acquired, side of body (left or right), age, body weight, gender, neuter status, and breed. Dogs were categorized as dachshund or non-dachshund, as well as chondrodystrophic or non-chondrodystrophic, and the effect of those groups on lymph node characteristics explored. Breeds categorized as chondrodystrophic included Bassett Hound, Beagle, Bichon Frise, Cardigan Welsh Corgi, Cocker Spaniel, Dachshund, English Bulldog, French Bulldog, Lhasa Apso, Miniature Poodle, Miniature Schnauzer, Pekingese, Pug, Shih Tzu and Toy Poodle.^{10,11} For evaluation of the effect of chondrodystrophic status dogs identified as mixed or cross breeds were not included in the analysis.

Potential animal characteristics were explored to identify key factors that might be important for partitioning the reference population before calculation of reference intervals.⁹ Initially the associations between potential partitioning factors and lymph node characteristics (size and x-ray attenuation) for one randomly selected lymph node from each dog were examined using appropriate statistical tests and univariate analysis. A priori, weight was considered as the most biologically relevant characteristic which might affect lymph node size characteristics to be considered for partitioning the related reference intervals. For continuous independent variables, association was examined with linear regression. For categorical independent variables, association was examined with the student t-test or analysis of variance tests; where data was non-normally distributed association was examined with the Wilcoxon rank sum test or Kruskal-Wallis rank test. Linearity of relationship between independent and outcome variables was assessed with inclusion of squared values for the independent variable. Variables that were potentially associated with each lymph node characteristic ($p < 0.2$) were included in multivariate linear regression analysis. All statistical analyses were completed by an analytical epidemiologist using a commercial statistics program (Stata 13, StataCorp, College Station, TX).

For practicality in use in partitioning the reference samples, age and weight were recoded into categories appropriate for the project and the data available. Lymph nodes were partitioned into subsets of samples based on factors identified as significantly associated with multiple lymph node characteristics following multivariate analysis. For the calculation of reference intervals, measurements from both lymph nodes from all dogs were included. Reference intervals were calculated from 95% percentiles of included lymph node characteristics where greater than 120 samples were available. Confidence intervals (90%) for the reference interval estimates were calculated. Where more than 40 and less than 120 samples were available, robust estimators were used to calculate the reference intervals.⁸ Dixon's range statistic was used to detect potential outliers.⁸ Reference intervals, confidence limits for intervals, and outlier detection statistics were calculated using MedCalc Statistical Software version 17.4 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2017).

4.4 Results

The presumptive diagnosis that led to CT scanning was categorized as intervertebral disc herniation (n = 152), vascular abnormality (e.g. portosystemic shunt) (n = 13), trauma (n = 9), gastrointestinal disease (including liver, spleen, and pancreas) (n = 8), genitourinary abnormality (e.g. ectopic ureter) (n = 7) and orthopedic abnormality (e.g. hip dysplasia) (n = 2). Nine clinically normal dogs were imaged for research purposes.

The median age of the dogs was 5 years (range, 4 months to 13 years). Twenty-five dogs imaged were under 2 years of age. The median body weight was 7.8 kg (range, 2.2 – 59.0 kg). There were 82 neutered females, 17 intact females, 85 neutered males and 16 intact males. Breeds included Dachshund (n = 72), mixed (n = 52), Shih Tzu (n = 7), Labrador Retriever (n = 6), French Bulldog (n = 5), Pekingese (n = 5), Cocker Spaniel (n = 4), German Shepherd (n = 4), Miniature Schnauzer (n = 4), Bichon Frise (n = 3), Lhasa Apso (n = 3), Poodle (n = 3), American Pitbull (n = 2), Beagle (n = 2), Boxer (n = 2), Chihuahua (n = 2), Golden Retriever (n = 2), Pug (n = 2) and 1 each of Airedale terrier, Alaskan Malamute, American Eskimo, Australian Cattle Dog, Australian Shepherd, Bassett Hound, Belgian Malinois, Border Collie, Bull Mastiff, Cardigan Welsh Corgi, Coonhound, English Bulldog, English Springer Spaniel, Karelian Bear Dog, Maltese, Shiba Inu, Siberian Husky, Toy Fox Terrier, Vizsla and Whippet.

Dogs were imaged in dorsal recumbency (n = 180), ventral recumbency (n = 15), left lateral recumbency (n = 4) and right lateral recumbency (n = 1). X-ray attenuation could not be measured using the PACS elliptical region-of-interest tool for 5 lymph nodes in 4 dogs on pre-contrast CT as the cross-sectional diameter of the nodes was too small. Slice thickness included 0.8mm (n = 55 dogs), 1.0mm (n = 101 dogs) and 2.0mm (n = 44 dogs).

Weight and age were not linearly associated with lymph node width, length, height or x-ray attenuation; a categorical variable was created to assess association for these two variables. Based on the range of the weights (2.2 to 59 kg) of dogs in the study, four weight groupings were created based on the range of weights and then the heaviest two groups were consolidated as very few dogs were in the heaviest group (n = 2). This created three weight groups: (1) ≤ 15 kg, (2) > 15 kg and ≤ 30 kg, and (3) > 30 kg. Age was categorized as non-adult (< 2 years) and adult (≥ 2 years). Breed was not evaluated for its effect on lymph node characteristics due to a lack of information for most breeds; of 148 dogs with identified breeds, 72 were dachshunds, and all other breed categories contained less than 10 dogs.

For univariate analysis, weight, age, gender, neuter status, chondrodystrophic versus non-chondrodystrophic (evaluated using the subset of dogs that did not include mixed breeds), dachshund versus non-dachshund (evaluated using the subset of dogs that did not include mixed breeds), and site of imaging were all potentially associated ($p < 0.20$) with more than one lymph node characteristic (Table 1). Classification as chondrodystrophic or as a dachshund were strongly associated with each other ($p < 0.0001$) and collinear; multivariate models were tested using both separately.

After multivariate analysis, width was significantly associated with weight, age, and being a dachshund ($p < 0.05$; Table 2). Length was significantly associated with weight, age, and gender ($p < 0.05$; Table 2). Height was significantly associated with weight, age, and site ($p < 0.05$; Table 2). The ratio of length to width was significantly associated with neuter status of the dog and site ($p < 0.05$; Table 2). X-ray attenuation was significantly associated with age and site ($p < 0.05$; Table 2). No significant interactions were detected in any of the models. Model assumptions were slightly violated for the models for length, width, height and the ratio of length to width. Transformation on the log scale improved fit but did not change model coefficients so models for untransformed results are presented. Model assumptions were met for the models for X-ray attenuation.

Only weight, age, and site were significantly associated with multiple lymph node characteristics. For calculation of lymph node characteristic reference intervals, data from both lymph nodes for each dog (where available) were included as lymph node measurements differed within dogs. Lymph nodes were partitioned into subgroups based on weight for calculating reference intervals for each lymph node characteristic. Lymph nodes were not partitioned into further subgroups based on age or site of collection for calculating reference intervals for each lymph node characteristic to limit creation of multiple subgroups with insufficient sample size.⁹

Calculated reference intervals for the middle and heaviest groups overlapped for most characteristics when three weight groups were used. For practicality of use in partitioning the reference samples, weight was recoded into two categories (≤ 15 kg and > 15 kg). For the purpose of calculating reference intervals, the reference samples were partitioned and divided into two subsets based on this weight categorization. X-ray attenuation was significantly different between sites, but not significantly different between different weight groups. For x-ray attenuation, one set of reference intervals was calculated, incorporating measurements from both

sites. X-ray attenuation was not partitioned by weight as there was no clinically significant difference between dogs weighing ≤ 15 kg and dogs weighing > 15 kg. For each of the lymph node characteristics and reference interval calculations, no potential outliers were detected.

The calculated upper and lower reference limits, and measured ranges, for width, height, length, length-to-width ratio and pre-contrast x-ray attenuation are presented in Table 3.

Suggestions for normal limits for width, height, length and length-to-width ratio are presented in Table 4.

4.5 Discussion

This study provides reference intervals for medial iliac lymph node size based on a large hospital-based population of dogs of varying weights and ages from two geographic sites. To the authors' knowledge, this is the first study to examine medial iliac lymph node size in an adequate number of dogs to report reference intervals. There is a lack of information on CT characteristics, including size, of the medial iliac lymph nodes in dogs; published data in dogs with no evidence of disease affecting the nodes are limited to two studies of a total of 39 adult dogs.^{3,6} Size is the only widely accepted imaging method for differentiating between normal and diseased lymph nodes.⁴ While size alone cannot be used to confirm metastasis, the results of this study can be used to raise the level of clinical suspicion and to recommend additional staging tests.

Previously reported mean CT size parameters for the medial iliac lymph nodes in clinically normal dogs fall within the measured range and the reference intervals described in our study.^{3,6} Direct comparison with previous studies is difficult as different methods were used. In the study of abdominal lymph nodes in 19 dogs, the mean reported sizes included more than one medial iliac lymph node on each side. We described characteristics of only the largest node on each side so that our reference intervals would represent the largest node on the right or left instead of the average of multiple nodes of various sizes. The study of 19 dogs reported that 76% (32/42) of medial iliac lymph nodes were elongated (short axis to long axis ratio equal to or less than 0.5), which is comparable to our findings. The HU in 100 abdominal lymph nodes in the previous study ranged from 20 to 52 before contrast administration, and this range falls within our measured range and calculated reference intervals.

After evaluating various factors that were hypothesized to impact lymph node CT size, only body weight was kept to partition the reference intervals. Age was significantly associated with some lymph node size parameters. We considered partitioning on weight and age characteristics. The American Society for Veterinary Clinical Pathology reference interval guidelines suggest not using partitions that create groups of less than 40 samples, and there were not enough young dogs in this study to meet those guidelines. In addition, the change in lymph node characteristics was small as age changed. It is possible that age should be considered in the development of reference intervals for lymph node size, however future studies would need to include more young dogs and those dogs would need to be from different size groups. Other

factors that were associated with only one measurement were not considered for partitioning as their effects on size were not consistent, not always very large and the sample sizes of subgroups would have been negatively impacted.

The study had fewer dogs over 15kg in weight, with a large weight distribution within that category. Robust estimates for the reference intervals for the length to width ratio provided an estimate of no practical use. The actual measured range was used for recommended reference intervals.

While we saw a difference in x-ray attenuation between the two sites, the overall range presented encompasses both sites as we did not feel it was useful to include reference ranges specific to a single site. This site difference may have arisen in part because of the tolerance level for CT number accuracy during scanner calibration, which may vary within ± 4 HU of zero for water.¹² As well, the scans at Colorado State University were all performed at 120 kVp, while the scans at the Western College of Veterinary Medicine varied from 100 to 135 kVp, and kVp will affect the measured HU. For these reasons, individual sites may elect to generate their own reference intervals for CT attenuation.

Some differences between the study population and a population of dogs presenting for assessment of potentially diseased lymph nodes may exist. Body weight was used instead of a frame measurement as frame measurements were not available retrospectively. If medial iliac lymph node size is associated with frame and not body condition, weight is not an ideal means of partitioning as the effect of frame on lymph node size would be missed. Body condition could be different between a population of dogs with cancer and the study population, and the reference intervals could be less accurate for an underweight cancer patient that weighs close to the 15kg partition. There was no ability to estimate an effect of breed on lymph node size as 49% of the dogs in this study with a clear breed description were Dachshunds. The median age of dogs with cancer is also likely greater than the median age of the study population.

While exclusion criteria were designed to exclude dogs with diseased nodes, no cytological or histological assessment of nodes was available to confirm that the nodes were normal. This may have led to the inclusion of some lymph nodes that were diseased. Another limitation of this study is that variation in measurements were potentially caused by differences in the angle of the dog relative to the CT acquisition plane, instead of differences in lymph node size.

In conclusion, this study describes medial iliac lymph node CT characteristics based on a population of 200 clinically normal dogs, providing useful reference intervals and suggested normal limits for CT interpretation. These results may also provide a basis for comparison with abnormal nodes in future studies.

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4.7 Tables

Table 4.7.1. Univariate Analysis Comparing Demographic Characteristics and Lymph Node Measurements for 400 Medial Iliac Lymph Nodes in 200 Clinically Healthy Dogs					
	WIDTH	LENGTH	HEIGHT	L:W^a	HU^b
Weight ^c	0.0001 ^e	0.0001 ^e	0.0001 ^e	0.02 ^f	0.28 ^g
Age ^d	<0.0001 ^h	0.0005 ^h	0.0001 ^h	0.35 ^h	0.03 ⁱ
Gender	0.007 ^h	0.004 ^h	0.13 ^h	0.63 ^h	0.06 ⁱ
Neuter Status	0.04 ^h	0.65 ^h	0.10 ^h	0.04 ^h	0.42 ⁱ
Chondrodystrophic (Yes/No)	<0.0001 ^h	<0.0001 ^h	<0.0001 ^h	0.53 ^h	0.66 ⁱ
Dachshund (Yes/No)	<0.0001 ^h	<0.0001 ^h	<0.0001 ^h	0.11 ^h	0.42 ⁱ
Site	0.17 ^h	0.72 ^h	0.01 ^h	0.01 ^h	0.0001 ⁱ

^a Length to Width Ratio

^b Hounsfield Units

^c Weight re-categorized to 3 groups

^d Age categorized to 2 groups, less than 2 years and 2 years and older

^e Not normally distributed; statistical test used was Kruskal-Wallis rank test

^f The statistical test used was linear regression

^g The statistical test used was the analysis of variance test

^h Not normally distributed; the statistical test used was the Wilcoxon Rank

ⁱ The statistical test used was the student's t-test

Table 4.7.2. Final Multivariable Linear Regression Models for the Associations Between Demographic Characteristics and Computed Tomographic Lymph Node Characteristics for 200 Medial Iliac Lymph Nodes in 200 Clinically Healthy Dogs

		B Coefficient	95% CI^a		p VALUE
			CI_L	CI_U	
Width (cm)	Model adjusted -R ² = 0.55				(n=148)
Weight	15 to 30 kg	2.75	1.69	3.81	<0.001
	> 30 kg	4.62	3.55	5.69	<0.001
	Reference category (<15 kg)				
Age	Mature	-1.8	-2.96	-0.64	0.003
Dachshund (Yes/No)		-0.94	-1.62	-0.27	0.006
Length (cm)	Model adjusted -R ² = 0.40				(n=200)
Weight	15 to 30 kg	8.05	5.21	10.89	<0.001
	> 30 kg	15.45	11.99	18.91	<0.001
	Reference category (<15 kg)				
Age	Mature	-4.69	-7.75	-1.62	0.003
Gender	Female	3.12	1.19	5.06	0.002
Height (cm)	Model adjusted -R ² = 0.47				(n=200)
Weight	15 to 30 kg	1.64	1.13	2.16	<0.001
	> 30 kg	3	2.38	3.62	<0.001
	Reference category (<15 kg)				
Age	Mature	-1.32	-1.87	-0.77	<0.001
Site	Site 2	0.49	0.14	0.85	0.006
Length to Width Ratio	Model adjusted -R ² = 0.05				(n=200)
Neuter Status	Intact	-0.79	-1.42	-0.15	0.016
Site	Site 2	-0.57	-1.04	-0.1	0.019
X-Ray Attenuation (Hounsfield Units)	Model adjusted -R ² = 0.10				(n=200)
Age	Mature	5.93	1.69	10.17	0.006
Site	Site 2	-5.99	-8.75	-3.22	<0.001

^aConfidence Interval, Confidence interval lower limit, confidence interval upper limit

Age categories: Young < 2 years, Mature 2 years and older

Note on n=148 – Final regression model only considered dogs where breed status was known

Table 4.7.3. Calculated Width, Height, Length, Length-to-Width Ratio and Pre-Contrast X-Ray Attenuation Intervals, and Measured Ranges, for 400 Medial Iliac Lymph Nodes in 200 Clinically Healthy Dogs					
	MEASURED	CALCULATED REFERENCE LIMITS			
	RANGE	L _l ^a	90% CI ^b	L _u ^c	90% CI
Width (cm)					
≤ 15 kg (n = 153 dogs)	0.19-1.31	0.24	0.20-0.26	0.89	0.82-1.20
> 15kg (n = 47 dogs)	0.32-1.65	0.27	0.19-0.35	1.49	1.40-1.59
Height (cm)					
≤ 15 kg	0.13-0.99	0.14	0.14-0.15	0.50	0.45-0.60
> 15kg	0.16-1.07	0.13	0.08-0.18	0.90	0.84-0.96
Length (cm)					
≤ 15 kg	0.48-5.40	0.70	0.60-0.80	3.07	2.80-3.50
> 15kg	0.80-7.20	0.79	0.38-1.19	4.50	4.08-4.91
Length-to-width ratio					
≤ 15 kg	1.07-11.58	1.5	1.3-1.6	8.0	7.2-10.0
> 15kg	1.05-7.37	0.2	-0.02-0.6	6.0	5.5-6.5
Attenuation (Hounsfield units)	1.50-56.21	7.5	4.2-10.4	49.3	47.3-51.5

^aLower Limit

^bConfidence Interval

^cUpper Limit

Table 4.7.4. Suggested Width, Height, Length and Length-to-Width Ratio Limits for Defining Normal Medial Iliac Lymph Nodes in Dogs, Based on 400 Medial Iliac Lymph Nodes in 200 Clinically Normal Dogs

Width

≤ 15 kg $\leq 0.9\text{cm}$

$> 15\text{kg}$ $\leq 1.5\text{cm}$

Height

≤ 15 kg $\leq 0.5\text{cm}$

$> 15\text{kg}$ $\leq 0.9\text{cm}$

Length

≤ 15 kg $\leq 3.1\text{cm}$

$> 15\text{kg}$ $\leq 4.5\text{cm}$

Length-to-width ratio

≤ 15 kg ≥ 1.5

$> 15\text{kg}$ Not available

5. Summary

The three studies performed added valuable information to the knowledge pool of both oncology and diagnostic imaging.

As with previously published lymphographic studies, we were not able to confirm a direct communication from the popliteal lymph node to the superficial inguinal lymph nodes. Potential contamination by perinodal leakage of contrast travelling via a known superficial lymphatic pathway was suspected in the five dogs of 50 in the study for which contrast uptake was seen in the superficial inguinal lymph nodes. However, a direct communication between the popliteal lymph node and the internal iliac and/or sacral lymph nodes happened more frequently than in previous studies, occurring in 13 of the 50 dogs in our study population. This pattern of drainage had been previously reported in one dog in lymphographic studies performed in the early 1900s and in two of 26 dogs and two of 19 dogs in lymphographic studies performed in the 1960s and 1970s. The relatively common finding of direct communication between the popliteal lymph node and the internal iliac lymph node was unexpected and is of clinical importance in staging of dogs with neoplastic disease in the popliteal lymph node. There were also cases of retrograde flow of contrast from the medial iliac lymph node to the internal iliac lymph node, and previously unreported flow between the left and right internal iliac lymph nodes that occurred in two of 50 study dogs. As with the direct communication between the popliteal lymph node and the internal iliac and/or sacral lymph nodes, the evidence of direct communication between the internal iliac lymph nodes is clinically important to staging and treatment of disease. Another unexpected result of this study was that the external iliac lymph node was not identified on the images of any of the dogs. These lymph nodes are known to be inconstant, often only occurring in one side, but are reported to be present in 36% of dogs.

The study population did not include any dogs with known disease of the lymphatic system. It is expected that lymphatic drainage patterns present in healthy dogs would also be present in those with neoplasia. What is unknown, and warrants future research, is to what extent

dogs with neoplastic processes may have development of, or opening of otherwise unidentified, lymphatic channels. Future research could include patients in which neoplasia has metastasized to the lymphatics. Use of smaller volumes of contrast or direct cannulation of the lymphatic vessels in future research is required to better understand potential communication between the popliteal lymph node and the superficial inguinal lymph node. This type of study would eliminate the concern of surface lymphatic drainage to the superficial inguinal lymph nodes from perinodal contamination with contrast.

Field margins for irradiation of normal-sized medial iliac lymph nodes without three-dimensional imaging varies by location and clinician. Treatment margins that are currently used in practice include treatment ventral to only the sixth and seventh lumbar vertebrae, which would have resulted in geographic misses of a portion of the medial iliac lymph node in 45 of 100 dogs in our study. Another margin which has been used to target the medial iliac lymph node when manually planning treatment is to limit the ventral margin of the field by the colon, with the goal of reducing normal tissue toxicity and causing side effects. In 48 of 100 dogs in our study population, there was overlap of the lymph node and the colon when viewed in the sagittal plane. The addition of concrete information regarding the location of normal-sized medial iliac lymph node with respect to the spine allows better treatment, thus having the potential to improve clinical outcome. Future research could include mapping with respect to bony anatomy of the lymph nodes caudal to the medial iliac lymph nodes that are also known to receive efferent flow from the pelvic limb in dogs.

Nodal size is the most widely accepted criterion to discriminate between normal and pathologic lymph nodes on CT examination, so a normal reference interval is required in order to best guide clinical decisions when staging and treating cancer-bearing dogs. Enlarged lymph nodes would raise the clinical suspicion of disease in the node, and a greater ability to distinguish between normal and abnormal lymph nodes will allow a more standardized approach to staging of patients with neoplastic disease that could drain to the medial iliac lymph nodes. Previous studies reporting the size of canine medial iliac lymph nodes on CT lacked case numbers or selection parameters to adequately formulate a de novo reference interval. The retrospective study allowed inclusion of a large number of dogs from two different institutions, but also introduced some selection bias. The population of dogs without cancer that had CT scans including the medial iliac lymph nodes may not accurately represent the population of interest.

Overall, many small and young dogs were included, especially Dachshunds. Many of larger dogs who had available CT scans including the medial iliac lymph nodes were tumour-bearing dogs and the images were therefore excluded from the study. In order to better represent the oncologic patient demographic, CT scans could be performed on healthy dogs matched to those bearing cancer. In our sample population, a cutoff of 15 kg was used since there were too few studies of larger breed dogs to have an appropriate sample size otherwise. This cutoff may need reconsideration if a greater number of larger dogs were enrolled in a future study. Consideration was given to using frame size rather than body weight to partition dogs in future research in order to account for dogs of varying body condition scores, but the retrospective nature of the study did not allow that. The funding required and concerns of anesthetizing or heavily sedating older patients may deter pursuit of future research.

Overall, clinical decisions can now be based on more complete information on the computed tomographic characteristics, location, and drainage patterns of lymph nodes in the pelvic canal and pelvic limbs in dogs.