Shear wave velocity measurements obtained in different regions are repeatable for presumed normal canine lymph nodes: a pilot study

Sophie Favril¹,², Hilde de Rooster¹,², Bart J.G. Broeckx³, Emmelie Stock⁴*, Katrien Vanderperren⁴*

¹ Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.
² Cancer Research Institute Ghent, Ghent, Belgium.
³ Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium.
⁴ Department of Medical Imaging and Small Animal Orthopaedics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.

* Shared last co-authors.

Corresponding author: Sophie Favril. Email address: sophiefavril@gmail.com

KEY WORDS
Intra- and interobserver study – Normal lymph nodes – Shear wave imaging

CONFLICTS OF INTEREST
The authors declare no commercial or financial conflicts of interest.

ABBREVIATIONS
BMI: body mass index; ICC: intraclass correlation coefficient; IQR: interquartile range; LN: lymph node; ROI: region of interest; SWE: shear wave elastography; SWV: shear wave velocity; US: ultrasonography.

**EQUATOR NETWORK GUIDELINE DISCLOSURE**

The authors followed ARRIVE reporting guidelines.

**PRESENTATION/PUBLICATION DISCLOSURE**

None of the study findings were previously presented at a scientific meeting or published in an abstract.
ABSTRACT

Shear wave elastography (SWE) has been applied as a noninvasive method for predicting regional lymph node (LN) metastases in human and veterinary patients. However, published studies describing standardized protocols and repeatability of this technique are currently lacking. The objective of this prospective pilot, observer agreement study was to determine whether different shear wave velocity (SWV) measurements obtained in different regions of presumed normal canine lymph nodes (LNs) would be repeatable. Two imagers consecutively performed shear wave elastography of submandibular, superficial inguinal and popliteal LNs in ten, clinically healthy adult dogs. Ten elastograms of each LN were acquired by each imager. In each adequate elastogram, three regions of interest (ROI) were placed in the softest and stiffest region of the LN. Additionally, one ROI was drawn covering the entire LN. In each ROI, mean, median and maximum SWVs were calculated. Mean values for the mean, median and maximum SWVs varied from 2.33 to 3.10 m/s, 2.32 to 3.10 m/s and 2.61 to 4.09 m/s, respectively. Intra- and interobserver agreements were acceptable. Superficial inguinal LNs demonstrated the highest intra- and interobserver agreement, followed by the popliteal and the submandibular LNs, respectively. Using the different measurements (mean, median or maximum SWVs) had no significant effect on the intra- and interobserver variability, neither did the region (softest, stiffest, or entire LN). Findings indicated that all evaluated measurements and regions could be used to obtain reliable elastography data of presumed normal canine LNs.
Similar to staging in human diagnostic oncology, regional lymph node (LN) assessment is an essential part of the staging procedure in canine cancer patients.\textsuperscript{1,2} Accurate evaluation of the LN status is of utmost importance for the prediction of outcome of many epithelial malignancies and the choice of optimal treatment strategy.\textsuperscript{1} However, currently used methodologies to evaluate LNs such as conventional ultrasonography (US) parameters (e.g. size, echogenicity, margin assessment and heterogeneity) and fine needle aspiration followed by cytologic evaluation have demonstrated only moderate sensitivity for the detection of metastases.\textsuperscript{3-6} To deal with the shortcomings of conventional LN assessment techniques, elastography has been introduced as a complementary US tool to evaluate the status of cervical LNs in human head and neck cancer patients.\textsuperscript{7} This method takes advantage of the changes in elasticity of soft tissues caused by specific physiological or pathological processes, including metastases.\textsuperscript{8} The majority of malignancies are composed of less elastic tissue compared to the surrounding normal tissue because of the stromal reaction induced by tumour cells, resulting in increased levels of collagen. Similarly, malignant LNs tend to be less compliant than normal LNs.\textsuperscript{9} Currently, two distinct types of elastography are available.\textsuperscript{10} Strain elastography measures the physical tissue displacement (so called ‘strain’) parallel to applied force, which is generally induced by freehand compression. This technique is dependent on the compression technique since excessive compression alters tissue stiffness, inducing nonaxial displacement, that can result in lower accuracy.\textsuperscript{9} In shear wave elastography (SWE), which is more recently developed, a different type of wave is generated because the tissue is mechanically stimulated by focused pulses. Shear waves move perpendicular
to the direction of the applied pressure. Measurement of the shear wave velocity (SWV; m/s) or elastic modulus (kPa) results in more accurate quantitative information about the tissue elasticity. This technique is less operator-dependent and a higher interobserver agreement is therefore to be expected. In human medicine, strain elastography and SWE demonstrated moderate to high sensitivity for predicting regional LN metastases in patients with head and neck cancer. However, published human studies differ in how they performed elastography of LNs. Particularly when SWE is used, often different velocities (i.e. mean versus maximum SWVs) are measured in different regions of the LNs (i.e. the stiffest region versus the whole LN), which hinders the comparison of the outcome of different studies. In addition, published cut-off values for the distinction between normal and metastatic cervical LNs vary as well. Furthermore, factors such as fasting, breathing, obesities, and positioning are known to cause variations in elastography data in abdominal organs. Thus, it remains currently unclear what is the most optimal protocol to be used. In veterinary medicine, only few research groups reported strain elastography and SWE as a diagnostic tool for the detection of metastatic LNs. Very recently, a research group published SWV values of LNs in healthy dogs. Various technical factors with potential effect on the reliability and reproducibility were investigated in abdominal organs. However, the evaluation of different velocity measurements (mean, median and maximum SWV) and different regions within the LN was not a topic of that research.

The objective of the current study was to determine whether different velocity measurements obtained in different regions of presumed normal LNs could be used to obtain reliable elastography data. Our hypotheses were that intra- and interobserver variability would be acceptable and that assessment of different anatomical LNs, different
velocity measurements, and different regions within the LNs would not have a significant
effect on the variability.

Materials & Methods

Selection and Description of Subjects

This study was a prospective, pilot, observer agreement study. All procedures were
approved by the local research ethical committee (Institutional Animal Care and Use
Committee; EC2019/29). Additionally, this project received approval of the deontological
committee of the Federal Public Service Health, Food Chain Safety and Environment for
the enrolment of non-purpose-bred dogs (DWZ/EV/19/1.15/50). All owners signed an
informed consent prior to enrollment of their dog into the study. Healthy client-owned
dogs of different breeds and over one year of age were considered eligible for enrolment
during the period of October 2019 and February 2020 at the Faculty of Veterinary
Medicine (Ghent University). All dogs were presented for reasons unrelated to the LNs.
For each dog, clinically healthy status was determined based on history and a thorough
physical examination indicating absence of symptoms of disease. Decisions for healthy
status and subsequent enrollment were based on a consensus of a board-certified
veterinary surgeon (H.D.R., European College of Veterinary Surgery [ECVS]), a board-
certified veterinary radiologist (K.V., European College of Veterinary Diagnostic Imaging
[ECVDI]) and a veterinarian with 7 years of clinical experience (S.F.).

Data Recording and Analysis
All dogs that were enrolled for the trial received sedation or anesthesia for other purposes (i.e. imaging of joints) to reduce motion artifacts on SWE.\textsuperscript{10} The sedative or anesthetic protocol was based on the attending anesthetist’s discretion. Two observers, (both board-certified veterinary radiologists (K.V. and E.S., ECVDI), independently but consecutively performed SWE of the LNs in all dogs, following an identical protocol. Observers used the same ultrasound machine (Philips Epiq7 (Brussels, Belgium) and the same ultra-broadband linear array transducer (Pure Wave eL18-4, Philips, Brussels, Belgium). The observers were not aware of the findings of the other observer. The order in which the LNs were imaged in each dog was randomized (https://www.randomizer.org).

The SWE protocol used by each observer was as follows. First, the fur overlying the superficial LNs of interest was clipped. To limit variations in the results caused by patient factors, all dogs were placed in a V-shaped cushion in dorsal recumbency, with the neck extended, and the front legs positioned in caudal direction. For the elastography of the popliteal LNs, the hind leg was raised in vertical direction by a technician. The acoustic gel was applied to the skin. B-mode US was performed to define the exact anatomical location, size, margins, shape and echogenicity of the different LNs. The transducer was placed perpendicular to the skin surface.\textsuperscript{25} Left and right sides of superficial inguinal and submandibular LNs were imaged in the longitudinal plane whereas the left and right sides of the popliteal LNs were imaged in the transverse plane. After B-mode US, the system was switched to SWE mode (ElastQ, software version 3.0.3., Philips, Brussels, Belgium), using the same probe and default elasticity settings of the m/s display scale (0-3.2). No standoff pad nor ECG was used. Patient and transducer positioning and handling were identical to that of B-mode US, including the generous use of acoustic coupling gel and gentle contact by the transducer on the skin surface. Care was taken not to exert pressure
on the transducer to avoid pressure artifacts. In the elastography colour maps, so-called elastograms, blue and red areas represented low (soft) and high (stiff) velocity regions, respectively. Images were acquired so that the LNs were centrally located in the elastograms. Interfering structures such as blood vessels were avoided. The elastograms were displayed alongside the grey-scale sonograms with a confidence map on the screen in real-time (“dual mode”). The confidence map provides an indication of quality across the elastogram, which assists the operator in obtaining measurements from regions with the highest shear wave quality. The confidence threshold was set at 50%, as recommended by the manufacturer (Philips, Brussels, Belgium), meaning that regions with confidence value of less than 50% were rendered transparent and not measurable. The operator froze the images that were deemed to be temporary stable. Each observer saved at least ten qualitative images of each LN, exceeding the minimum measurement number described in the guidelines of the World Federation for Ultrasound in Medicine and Biology.

**Image analyses**

All stored images were evaluated for image quality by a veterinarian with seven years of clinical experience (S.F.). If the colour map of the elastogram was not filling the image, it was excluded from further analysis. At least eight elastograms of the LN had to be adequate before the LN was included for further analyses. In each adequate elastogram, the veterinarian placed three circular regions of interest (ROIs) with a diameter of one mm on the softest (bluest) region in the LN. Care was taken to avoid overlap between the ROIs. Subsequently, three circular ROIs with a diameter of one mm were placed on the stiffest (reddest) region in the LN. Finally, a ROI was drawn manually covering the total
area of the LN (Figure 1). In each ROI, SWVs were generated by the software (mean, median, maximum SWV and interquartile range (IQR)). The IQR, reflecting variability of measurements, is equal to the difference between the upper and lower quartiles and is used to assess quality of the data. An IQR<30% suggests that a data set is adequate. Therefore only ROIs with velocity data with an IQR<30% were included for analyses. The veterinarian repeated this procedure for each elastogram.

Statistics

All statistical analyses were selected and completed by a statistician (B.B.), using statistical analysis freeware (R version 3.6.3, “Holding the Windsock”). The analyses were divided into four subparts: 1) evaluation of the intra- and interobserver agreement, 2) calculation of 95% reference intervals, 3) evaluation of systematic differences between the LN and the regions evaluated and 4) evaluation of the effect of age and sex on the values. For part 1, to evaluate the intra- and interobserver agreement, a random effects model was used per LN (popliteus, mandibular, inguinal) and per region (softest/stiffest region or whole LN) with as dependent variables the mean, median and maximum SWV, leading to a total of 27 models to allow a direct comparison further downstream. The random effect of the model was specified as image (for the softest and stiffest region only) within observer (1 or 2), nested within side (left/right), nested within dog (1 to 10). The residual variance and the residual variance together with the added variance by image for the region of the whole LN and the other two regions, respectively, were used to calculate the 95% intra-observer agreement. The added variance by observer was used to calculate the 95% interobserver agreement. The proportion of the intra- and interobserver agreement relative to the total variability was next compared in a simple linear model
with either the measured technique (\textit{i.e.} mean, median, maximum SWV), LN (mandibular, inguinal, popliteus) or region (softest region, stiffest region, whole LN) as independent variable. For part 2, the combination of the residual variance, the variance added by image (when applicable), observer, side and dog were used to calculate the 95% reference intervals per LN and per region. For part 3 and 4, a linear mixed model with the same random effect as specified before, but with LN, region, age or sex as fixed effect, was used. A likelihood ratio test was used to evaluate the significance of the fixed effects. Significance was set at $\alpha \leq 0.05$.

Results

Sixty presumed LNs of the ten clinically healthy adult dogs were included in analyses (detailed descriptions provided in Supplement 1). Of these, 13 LNs scans were excluded because elastography could not be performed (Supplement 2). B-mode ultrasonographic characteristics were within normal ranges for all included LNs.\textsuperscript{28} In total, 19656 measurements were performed on 6552 images: at least eight qualitative images of each LN scan were used for further analyses. All LNs were located at a depth between 3 and 11 mm (i.e., all ROIs were placed at similar depths).

Intra- and interobserver agreements were acceptable. Data demonstrating the 95\% limits of agreement (intra-observer variability, interobserver variability, and total variability) for each LN and each measured region are provided in Table 1. The proportion of the intra- and interobserver variability relative to the total variability is also depicted in that table. For all measured regions and all measured LNs, 45\% (for the whole superficial inguinal LN) up to 80\% (for the softest region in the submandibular LN) of the total
variability was caused by intra-observer variability, and 85% (for the whole superficial inguinal LN and softest region in the popliteal LN) up to 100% (for the whole submandibular LN) of the total variability was caused by the interobserver variability. The choice of measurement (mean, median or maximum SWVs) had no statistically significant effect on the intra- nor interobserver variability, neither did the evaluated region (most elastic, stiffest, or whole LN).

There was a significant difference between the evaluated LNs (submandibular, inguinal and popliteal) on the intra- and interobserver agreement scores (p<0.001). The superficial inguinal LNs demonstrated the highest intra- and interobserver agreements, whereas the lowest agreements were observed for the submandibular LNs. Mean values for the mean, median and maximum SWVs varied from 2.33 to 3.10 m/s, 2.32 to 3.10 m/s and 2.61 to 4.09 m/s, respectively. The 95% reference intervals and an overview of all mean values of the mean, median and maximum SWVs of each LN and each region is provided in Table 2. Mean, median and maximum SWVs were significantly different between the different LNs (p<0.001) and regions (p=0). Age and sex did not have a significant effect on the SWVs (p>0.05).

Discussion

This pilot study was intended to evaluate reproducibility of elastography features of normal LNs in healthy dogs using SWE and to provide reference interval values for future studies. This study focused on the influence of two technical factors (measurements and regions) because no standardized protocol for these factors has been described so far. This study demonstrated that the different evaluated measurements, the mean, median and maximum SWVs, can be used to collect reliable elastography data in the different LNs.
Furthermore, ROIs can be placed in the softest or stiffest region of the LN, and also a ROI covering the entire LN can be used to generate reliable elastography measurements in normal LNs.

Various technical and patient factors have been demonstrated to influence SWE data of *f.e.* liver, prostate, and breast, such as motion, patient positioning, tissue approach, image quality and measuring depth.\(^8\)\(^2\)\(^5\)\(^2\)\(^6\) So far, there are no guidelines available for SWE of LNs, but in an attempt to minimize variations in SWE data caused by these factors, all dogs were anaesthetized and an identical positioning and tissue approach (transducer perpendicular to the skin and identical approach to LNs) was pursued in this study. Care was taken not to put pressure on the transducer during the procedure, and at least eight qualitative elastograms had to be acquired before the LN was included for analysis. The influence of measuring depth was suspected to be low because of the enrolment of superficial LNs only.

A recent publication described SWVs of various canine organs including normal superficial LNs.\(^2\)\(^4\) They reported mean SWVs of 1.62 ± 0.07 m/s and 1.55 ± 0.1 m/s for the submandibular and inguinal LNs respectively, based on a ROI covering the largest part of the LN.\(^2\)\(^4\) Their high intraclass correlation coefficient (>0.9) supported our findings that SWE is a reliable technique to evaluate tissue elasticity. On the other hand, their mean values for SWVs were remarkably lower than our mean values for these nodes. Moreover, no significant difference in SWVs between LNs was noted by the other researchers and all LNs showed a uniform blue (elastic) colour in the elastogram.\(^2\)\(^4\)

Conversely, SWVs were significantly different between LNs, and even between different regions in our study. Other US device and software tools were used for this previous study, making direct comparison of the results challenging because a known limitation of SWE is the lack of uniformity of commercial system design and settings.\(^1\)\(^1\) Furthermore,
different positioning and scan approach (right lateral recumbency compared to dorsal recumbency in our trial) and the use of anesthesia or not might all be factors causing different results.\textsuperscript{10}

Axillary and inguinal LNs in dogs with mammary cancer were evaluated using SWE in another study.\textsuperscript{20} Mean SWVs of $1.91 \pm 0.44$ m/s, $2.29 \pm 0.19$ m/s and $2.99 \pm 0.64$ m/s for the ROIs of entire normal, reactive and metastatic superficial inguinal LNs, respectively, were reported whilst in our trial, a mean SWV for the whole superficial inguinal LNs of $2.55 \pm 0.99$ m/s was found, with a 95% reference interval of $1.56 – 3.53$ m/s. The mean SWV of the metastatic LNs in the previous trial is clearly situated within our 95% reference interval. One can question whether the difference in mean SWVs of the entire LNs between normal and metastatic LNs is large enough to use as a marker for differentiation. Clinical trials evaluating SWVs in the entire and stiffest part of suspected LNs in dogs with cancer will reveal whether the SWVs of metastatic LNs fall outside the reference intervals of healthy LNs defined in our trial. However, based on the results obtained in the current study, the intra- and interobserver variability (presented as the 95% limits of agreement in Table 1) covers 43% ($0.45/(2.99 – 1.91)$) and 80% ($0.84/(2.99 – 1.05)$) of the difference between the metastatic and normal LNs, respectively. If the difference observed in the previous study\textsuperscript{20} between normal and metastatic LNs is indeed similar to what we would observe, it is clear that the variability should be kept as low as possible. Practically, this also means limiting the number of operators, ideally to one.

In the human literature, there seems to exist a selection bias in the published elastography studies, since elastography has been always deployed as an additional US tool only when US findings revealed abnormal LNs, thereby resulting in samples that included a high portion of malignancies. Therefore, it is important to gather knowledge on elastography
features of normal structures. In 2017, a research group published minimum, maximum, and mean SWE features of normal cervical LNs in 178 volunteers. They reported a mean shear modulus of 9.5 ± 4.6 kPa (equivalent to a mean SWV of 3.2 ± 1.5 m/s) after measurement of the entire LNs. Similar to our results, there was no systematic effect of sex and age on the elastography features; neither did body mass index (BMI) have a systematic effect on the features, except for minimum SWVs that significantly decreased with increasing BMI. Moreover, a trend towards a decreased tissue stiffness with increasing age was noticed. A larger population of dogs, including dogs in different life stages, would need to be evaluated in order to explore this topic further. More recently, another group investigated SWVs in cervical LNs of 30 healthy humans in an intra-and interobserver study. SWVs of a ROI covering a large homogeneous area within the LNs were measured. They described a median SWV of 1.82 m/s and 1.65 m/s for both observers. Similar to our results and the recently published SWV data in normal canine LNs, there exists a remarkable difference between the reported SWVs in the human studies. Furthermore, the more recent human study described an excellent repeatability (intra-class correlation coefficient >0.90) but a low inter-operator reproducibility (ICC:0.4). A direct comparison to our results is not possible as the variability is not described in the actual measurement units, which is the case in our study. However, similar to our study and in agreement with expectations, the repeatability is better than the reproducibility.

So far, SWE has mainly been investigated in human head and neck cancer patients. Velocities in the entire and the stiffest region of the regional cervical LNs were assessed and the results demonstrated heterogeneity in the cut-off values, particularly for mean SWV values that were measured in the entire LN, ranging from 1.94 m/s to 3.4 m/s.
In clinical cases, it is interesting to determine whether selection of a ROI covering the total area of the LN of interest or only the stiffest region might reveal the most useful information. Our study demonstrated that, in normal LNs, the choice of ROI placement did not influence the elastography results. However, this finding will not necessarily remain valid in case of LN pathology because local invasion can result in a focal region with increased stiffness, without complete deformation of the lymphatic parenchyma. Further investigation in oncology patients is therefore triggered.

There are some limitations to the use of elastography in veterinary medicine. First, it seems a challenge to obtain high-qualitative elastograms. Elimination of movement due to respiration, due to pulsation caused by blood vessels or limited cooperation of patients can cause artifacts that result in unreliable data. The animal should therefore ideally always be sedated or anesthetized. Another limitation of shear wave imaging is that the precise implementation of SWE technology differs among US system manufacturers and that not only different techniques are currently used to measure SWVs, but also various software techniques are involved, such as acoustic radiation force impulse elastography and supersonic shear elastography, which might implicate that the reported reference intervals in this trial are only applicable for the used software.

A limitation of this study is that, although an enormous number of images were obtained and evaluated in ten dogs, a larger number of dogs would be needed in order to set-up reference intervals for the different LNs. Furthermore, only a selected group of superficial LNs were examined. Ideally, SWV data of other superficially located LNs should be gathered to also define reference intervals for those LNs. Additionally, it proved to be more difficult to obtain sufficient elastograms of the submandibular LNs, because of
superposition of the nearby located mandibula and a limited contact area between the transducer and the skin of the dog. Another limitation is that the LNs were deemed normal based on palpation and B-mode US only. Ethical restraints prohibited removal and subsequent histopathology of the LNs. Yet histopathology might reveal important information about correlation between different SWV measurements and histological features of the LN. Finally, ideally, elastography data of dogs in other life stages should be obtained as well to evaluate the effect of increasing age on elasticity, similar to the observations in people.29

To conclude, this study demonstrated that, in normal LNs, ROIs in different regions can be used to evaluate tissue elasticity using SWE. In addition, different measurements (mean, median, and maximum SWVs) are suitable to obtain reliable data; preliminary reference intervals are provided. While intra- and interobserver variability is acceptable, it is clear that, ideally, the number of operators in clinical trials is limited to reduce the technical variability. Although clinical trials in human oncology and one clinical trial in veterinary oncology demonstrated significant differences between the SWVs of normal and metastatic LNs, larger clinical trials are warranted to define whether SWE can differentiate between non-metastatic and metastatic LNs and which area should preferentially be examined to optimize the accuracy of this technique for the identification of metastatic LNs.
LIST OF AUTHOR CONTRIBUTIONS

Category 1:

(a) Conception and design: Favril, Vanderperren, de Rooster, Stock
(b) Acquisition of data: Favril, Vanderperren, Stock
(c) Analysis and interpretation of data: Favril, Broeckx, de Rooster

Category 2:

(a) Drafting the article: Favril
(b) Revising article for intellectual content: de Rooster, Vanderperren, Stock, Broeckx

Category 3

(a) Final approval of completed article: Favril, de Rooster, Vanderperren, Stock, Broeckx

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Table 1. An overview of the 95% limits of agreement for the intra-, inter- and total variability for each measured region (most elastic, stiffest and whole region of the lymph node) in each type of lymph node (submandibular, superficial inguinal, and popliteal).

<table>
<thead>
<tr>
<th>LN</th>
<th>Region (number of observations)</th>
<th>Intra-mean**</th>
<th>Inter-mean**</th>
<th>Tot</th>
<th>Intra-mean***</th>
<th>Inter-mean***</th>
<th>Tot</th>
<th>Intra-med**</th>
<th>Inter-med**</th>
<th>Tot</th>
<th>Intra-med***</th>
<th>Inter-med***</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>E (n=762)</td>
<td>± 0.53</td>
<td>± 0.66</td>
<td>± 0.67</td>
<td>79</td>
<td>± 0.67</td>
<td>98</td>
<td>± 0.54</td>
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<td>± 0.67</td>
<td>80</td>
<td>± 0.67</td>
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<tr>
<td>M</td>
<td>S (n=762)</td>
<td>± 0.53</td>
<td>± 0.72</td>
<td>± 0.73</td>
<td>73</td>
<td>± 0.73</td>
<td>99</td>
<td>± 0.55</td>
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<td>M</td>
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<td>± 0.63</td>
<td>± 0.64</td>
<td>66</td>
<td>± 0.64</td>
<td>98</td>
<td>± 0.45</td>
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<td>± 0.67</td>
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<td>± 0.67</td>
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<tr>
<td>I</td>
<td>E (n=1038)</td>
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<td>± 0.82</td>
<td>± 0.94</td>
<td>49</td>
<td>± 0.94</td>
<td>87</td>
<td>± 0.47</td>
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<td>87</td>
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<tr>
<td>I</td>
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<td>± 0.61</td>
<td>± 0.97</td>
<td>± 1.12</td>
<td>54</td>
<td>± 1.12</td>
<td>86</td>
<td>± 0.61</td>
<td>± 0.98</td>
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<td>± 1.12</td>
<td>86</td>
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<tr>
<td>I</td>
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<td>± 0.99</td>
<td>45</td>
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<td>85</td>
<td>± 0.47</td>
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<td>± 1.00</td>
<td>85</td>
</tr>
<tr>
<td>P</td>
<td>E (n=1002)</td>
<td>± 0.51</td>
<td>± 0.72</td>
<td>± 0.85</td>
<td>61</td>
<td>± 0.85</td>
<td>85</td>
<td>± 0.52</td>
<td>± 0.72</td>
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<td>61</td>
<td>± 0.85</td>
<td>85</td>
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<tr>
<td>P</td>
<td>S (n=1002)</td>
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<td>± 0.82</td>
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<td>± 0.86</td>
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<td>± 0.54</td>
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<td>± 0.80</td>
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<td>± 0.72</td>
<td>± 0.81</td>
<td>57</td>
<td>± 0.81</td>
<td>90</td>
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</tbody>
</table>
The region refers to the exact location where the regions of interest were placed within the lymph node to define the shear wave velocities (in the most elastic region, stiffest region or the whole lymph node). Data are expressed in m/s and represent 1.96 x the standard deviation. The proportion of the intra- and interobserver variability relative to the total variability, provided in percentages.

Abbreviations: E, most elastic region of the lymph node; I, superficial inguinal lymph node; LN, lymph node; M, submandibular lymph node; max, maximum, med, median; P, popliteal lymph node; S, stiffest region; tot, total, W, whole lymph node.
Table 2. An overview of the mean shear wave velocities and 95% reference intervals for each region within each type of lymph node.

<table>
<thead>
<tr>
<th>LN</th>
<th>Region* (number of observations)</th>
<th>Mean of the mean SWV</th>
<th>95% RI mean SWV</th>
<th>Mean of the median SWV</th>
<th>95% RI median SWV</th>
<th>Mean of the maximum SWV</th>
<th>95% RI maximum SWV</th>
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<td>2.38</td>
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<td>1.84 – 3.38</td>
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<td>2.35 – 3.81</td>
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All data are expressed in m/s.
The region refers to the exact location where the regions of interest were placed within the lymph node to define the shear wave velocities (in the most elastic region, stiffest region or the whole lymph node).

Abbreviations: E, most elastic region; I, superficial inguinal lymph node; LN, lymph node; M, submandibular lymph node; P, popliteal lymph node; RI, reference interval; S, stiffest region; SWV, shear wave velocity; W, whole lymph node.
FIGURE LEGENDS

Figure 1. Ultrasonography images of a popliteal lymph node in dog 6 (dorsal recumbency with hind leg raised in vertical direction, eL18-4, transverse plane). Image (A) represents a grey-scale image in which the lymph node is delineated by a red dotted line. Images (C) and (E) represent grey-scale images covered by a confidence colour map. Reliable elastography measurements can be performed in tissue that is located in the green area. Images (B, D, F) illustrate elastography colour maps, so called elastograms, with regions of interest (ROIs) placed in different regions. The colour scale bar in the right upper side represents a colour code for the elasticity in tissue in which blue areas represent soft tissue and red areas represent stiff tissue. In image (B) and (D), three circular ROIs are placed in the softest (B) and stiffest (D) region of the lymph node. In image (F), one ROI is placed covering the entire parenchyma of the lymph node. Mean, median and maximum shear wave velocities were calculated for each ROI by the ultrasound machine’s software and are shown on the right side of the images.