

Ultrasonographic Characterization of Splenic Height in Clinically Healthy Dogs Across Different Body Weight Categories

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Abstract

Accurate ultrasonographic assessment of splenic dimensions is important when evaluating dogs for possible splenomegaly, but standardized canine data accounting for body-weight variation remain limited. This study characterized ultrasonographic splenic height in clinically healthy dogs and evaluated differences by body-weight categories, sex, and reproductive status. Forty-two clinically healthy dogs underwent physical examination, hematological and serum biochemical screening, and complete abdominal ultrasonography. Sagittal and transverse splenic heights were measured at the level of the splenic hilum. Dogs were classified into study-specific body-weight (<10 kg, 10–20 kg, and >20 kg). Mean $\pm$ SD sagittal and transverse heights were 15.43 ± 4.56 mm and 15.96 ± 4.76 mm, respectively. The mean difference between transverse and sagittal measurements was 0.53 mm (95% CI: 0.01–1.04 mm; paired t-test, $P = 0.045$), with transverse values being systematically higher; the Wilcoxon signed-rank test yielded $P = 0.018$. Splenic height differed among the three body-weight categories in both planes (both $P < 0.001$). No statistically significant unadjusted differences were detected by sex or reproductive status; in an exploratory model adjusted for body-weight category, sagittal and transverse heights were lower in males than in females (-2.99 mm, $P = 0.001$; -2.25 mm, $P = 0.041$), whereas reproductive status remained non-significant. Continuous body weight was strongly correlated with height (Spearman $\rho = 0.83$ and 0.79). These findings provide preliminary descriptive data rather than validated reference intervals or diagnostic thresholds. The modest sample size and limited breed diversity warrant cautious interpretation and further evaluation in larger populations.

Keywords: Canine spleen; ultrasonography; splenic height; body weight

List of Abbreviations

CI, confidence interval; ICC, intraclass correlation coefficient; SD, standard deviation

Introduction

The spleen is a secondary lymphoid organ which is involved in filtering the blood and generating immune responses against antigens in the blood [1]. It is the largest lymphoid organ in the body, and unlike other lymph nodes, is perfused by blood rather than lymph. The spleen is the principal

organ specialized for filtering the blood. The body of the spleen is surrounded by a connective tissue capsule and smooth muscle cells [2].

Anatomically, the spleen is a lymphoid structure closely associated with the circulatory system. This organ is attached to the stomach by the gastrosplenic ligament; blood vessels enter splenic parenchyma at the splenic hilum, the splenic vein carries blood from the spleen to the portal vein. The spleen is located on the left side of the cranial abdomen, usually along the greater curvature of the stomach in dogs. The exact location of the spleen varies depending on the extent of gastric distension and the size of adjacent abdominal organs. The head of the spleen is usually located below costal margin and its body and tail extend along the left abdominal wall or across the abdomen. The spleen is located lateral and caudal to the stomach and ventral or lateral to the left kidney. When the spleen is enlarged, it may cross the ventral midline or extend caudally toward the bladder [3]

On ultrasonography examination, the normal canine spleen is often described as hyperechoic compared to the liver and renal cortex. The branches of the splenic vein are echogenic tubular structures within the splenic parenchyma and exit the spleen at the hilum. The splenic artery is generally not visible unless color Doppler imaging is applied [4].

Splenomegaly can be physiologically and breed-specific, especially in German Shepherds and Greyhounds, or it can be due to various diseases and disorders such as congestion, hematoma, hyperplasia, immune-related inflammation, etc. Moreover, its size can be reduced as a result of dehydration and shock [5].

Clinical signs in dogs with splenomegaly are usually vague and nonspecific. Clinical signs of the disorders that are related to specific splenic enlargement include: splenic torsion and primary splenic hyperplasia. Other signs associated with splenomegaly are those resulting from the hematologic consequences of splenomegaly and include spontaneous hemorrhage, mucosal pallor, and fever. Ultrasonography is extremely helpful in the evaluation of patients with splenomegaly or splenic masses. Ultrasound-guided fine needle aspiration (FNA) or biopsies can aid in differential diagnosis [6].

Ultrasonographic assessment of the spleen is very important. In dogs with splenomegaly, the splenic margins are rounded and extended more caudally and toward the right side of the abdomen.

However, the detection of homogeneous splenomegaly is challenging, as its ultrasonographic evaluation is inherently subjective in dogs. Homogenous splenomegaly is common in dogs being sedated with acepromazine, thiopental [7], ketamine, and diazepam [8]. However, it may occur secondary to extramedullary hematopoiesis, infectious diseases, splenic torsion, splenic vein thrombosis, or malignant infiltration, such as that seen in lymphoma and mast cell tumors, as described in dogs and cats [9–11].

In a recent study, the average spleen height in cats was reported to be 8.2 mm (SD 1.4 mm) and width 26.7 mm (SD 4.4 mm) on transverse images [12]. Another study reported the average normal spleen height in cats to be 7.1 mm, with a range of 5.1 to 9.1 mm. It was also suggested that a spleen height greater than 9.1 mm may indicate splenomegaly [13].

Despite significant advancements, further research is needed to establish ultrasonographic splenic dimensions and factors influencing the normal splenic size in healthy dogs. This further highlights the importance of conducting comprehensive studies based on accurate ultrasonographic methods. The present study was designed to provide a detailed evaluation of ultrasonographic dimensions of the spleen, including measurements of splenic height in sagittal and transverse view in 42 healthy dogs, and also to investigate the impacts of body weight, sex and reproductive status on normal splenic size in healthy dogs. Intra- and inter-observer reliability of the measurements was also assessed.

Results

Forty-two dogs met all predefined eligibility criteria and were included in the final analysis, comprising 19 males (45.2%) and 23 females (54.8 %); 23 dogs (54.8%) were intact and 19 (45.2%) were neutered/spayed. By body weight, 16 dogs (38.1%) weighed <10 kg, 14 (33.3%) weighed 10–20 kg, and 12 (28.6%) weighed >20 kg. Age ranged from 10 months to 11 years, with a mean $\pm$ SD of 43.74 ± 30.75 months (approximately 3.64 ± 2.56 years-) and a median of 33 months (2.75 years). Mean $\pm$ SD body weight was 5.85 ± 1.72 kg in the <10 kg category, 12.51 ± 1.49 kg in the 10–20 kg category, and 30.25 ± 8.25 kg in the >20 kg category (overall range, 3.6–45.0 kg). Sex and reproductive status were unevenly distributed across the weight categories (Table 4).

All primary clinical analyses were based on the mean of the three repeated measurements obtained by Observer 1 for each dog and measurement plane.

Normality Assessment:

The Shapiro–Wilk test indicated that overall sagittal splenic height was not normally distributed ($P = 0.032$), whereas transverse splenic height showed no evidence of non-normality ($P = 0.074$). Within the <10 kg and >20 kg categories, neither measurement showed evidence of non-normality (all $P > 0.05$); in the 10–20 kg, both sagittal and transverse measurements departed from normality ($P = 0.021$ and $P = 0.020$, respectively). The distribution of within-dog differences between transverse and sagittal measurements showed no evidence of non-normality (Shapiro–Wilk $P = 0.383$).

Comparison between Splenic Measurement Views:

Analysis of splenic height in sagittal and transverse views revealed mean values of 15.43 ± 4.56 mm (95% CI: 14.01–16.85) and 15.96 ± 4.76 mm (95% CI: 14.48–17.44), respectively.

The mean difference between transverse and sagittal measurements was 0.53 mm (95% CI: 0.01–1.04 mm; paired t-test, $P = 0.045$), with transverse values being systematically higher. Because the paired differences showed no evidence of non-normality, the paired t-test was used as the primary comparison ($P = 0.045$); the Wilcoxon signed-rank test was retained as a sensitivity analysis ($P = 0.018$).

Effects of Body Weight:

The Kruskal–Wallis test showed differences among the three study-specific body-weight categories for sagittal splenic height ($H = 28.45$, $df = 2$, $P < 0.001$; $\eta^2_H = 0.678$) and transverse splenic height ($H = 23.84$, $df = 2$, $P < 0.001$; $\eta^2_H = 0.560$). Dunn pairwise comparisons with Holm adjustment showed differences between all three pairs of categories. For sagittal height, adjusted P values were 0.0039 (<10 kg vs 10–20), <0.001 (<10 kg vs >20 kg), and 0.025 (10–20 kg vs >20 kg). For transverse height, adjusted P values were 0.026, <0.001 , and 0.015 for the same comparisons, respectively (Table 1). Continuous body weight was strongly and positively correlated with sagittal (Spearman $\rho = 0.83$, $P < 0.001$) and transverse splenic height ($\rho = 0.79$, $P < 0.001$).

Table 1. Ultrasonographic measurements of splenic height stratified by body-weight category (all values in mm).

Measurement	Weight	N	Mean	SD	SEM	95% CI	Min.	Max.
Sagittal height	<10 kg	16	11.56	2.22	0.56	10.38– 12.75	7.97	14.61
	10–20 kg	14	15.53	2.06	0.55	14.34– 16.72	13.63	20.62
	>20 kg	12	20.49	4.01	1.16	17.94– 23.03	14.63	26.10
	Total	42	15.43	4.56	0.70	14.01– 16.85	7.97	26.10
Transverse height	<10 kg	16	12.27	3.00	0.75	10.67– 13.86	8.33	17.26
	10–20 kg	14	15.80	2.75	0.73	14.21– 17.39	12.33	22.70
	>20 kg	12	21.08	3.84	1.11	18.64– 23.52	15.10	26.37
	Total	42	15.96	4.76	0.73	14.48– 17.44	8.33	26.37

N: number of dogs; Mean: arithmetic mean; SD: standard deviation; SEM: standard error of the mean; 95% CI: 95% confidence interval; Min.: minimum value; Max.: maximum value. All measurements are expressed in millimeters (mm). Kruskal–Wallis test: sagittal height $H = 28.45$, $df = 2$, $P < 0.001$, $\eta^2_H = 0.678$; transverse height $H = 23.84$, $df = 2$, $P < 0.001$, $\eta^2_H = 0.560$. All pairwise comparisons remained significant after Holm adjustment.

Effects of Sex and Reproductive Status:

In unadjusted comparisons, no statistically significant differences in splenic height were found based on sex or reproductive status. Independent-samples t-tests (Welch) showed no significant effect of sex on sagittal spleen height (mean difference, male minus female, -1.79 mm, 95% CI -4.49 to 0.91 ; $P = 0.187$) or transverse spleen height (mean difference -1.29 mm, 95% CI -4.14 to 1.56 ; $P = 0.366$). Similarly, reproductive status had no significant effect on sagittal spleen height (mean difference, intact minus neutered/spayed, -0.66 mm, 95% CI -3.52 to 2.20 ; $P = 0.645$) or transverse spleen height (mean difference -0.51 mm, 95% CI -3.47 to 2.45 ; $P = 0.730$) (Tables 2 and 3). Mann–Whitney U tests gave concordant results (sex: $P = 0.404$ for sagittal and 0.762 for transverse height; reproductive status: $P = 0.363$ and 0.561 , respectively).

Table 2. Ultrasonographic measurements of splenic height stratified by sex (all values in mm).

Measurement	Sex	N	Mean	SD	SEM	95% CI
Sagittal height	Male	19	14.45	2.97	0.68	13.02–15.88
	Female	23	16.24	5.48	1.14	13.87–18.61
Transverse height	Male	19	15.26	3.21	0.74	13.71–16.80
	Female	23	16.54	5.75	1.20	14.06–19.03

N: number of dogs; Mean: arithmetic mean; SD: standard deviation; SEM: standard error of the mean; 95% CI: 95% confidence interval. All measurements are expressed in millimeters (mm).

Unadjusted Welch's independent-samples t-test: sagittal height, mean difference (male – female) = -1.79 mm (95% CI -4.49 to 0.91), $P = 0.187$; transverse height, mean difference = -1.29 mm (95% CI -4.14 to 1.56), $P = 0.366$.

Table 3. Ultrasonographic measurements of splenic height stratified by reproductive status (all values in mm).

Measurement	Reproductive status	N	Mean	SD	SEM	95% CI
Sagittal height	Intact	23	15.14	4.79	1.00	13.06–17.21
	Neutered/spayed	19	15.79	4.37	1.00	13.69–17.90
Transverse height	Intact	23	15.73	5.21	1.09	13.48–17.98
	Neutered/spayed	19	16.24	4.27	0.98	14.18–18.30

N: number of dogs; Mean: arithmetic mean; SD: standard deviation; SEM: standard error of the mean; 95% CI: 95% confidence interval. All measurements are expressed in millimeters (mm).

Unadjusted Welch's independent-samples t-test: sagittal height, mean difference (intact – neutered/spayed) = –0.66 mm (95% CI –3.52 to 2.20), P = 0.645; transverse height, mean difference = –0.51 mm (95% CI –3.47 to 2.45), P = 0.730.

Because sex and reproductive status were unevenly distributed across weight categories (Table 4), exploratory linear models including body-weight category, sex, and reproductive status simultaneously were fitted (Table 5). After adjustment, males had lower splenic height than females (sagittal: –2.99 mm, 95% CI –4.71 to –1.27, $P = 0.001$; transverse: –2.25 mm, 95% CI –4.41 to –0.10, $P = 0.041$), whereas reproductive status was not associated with splenic height (sagittal: 0.20 mm for neutered/spayed vs intact, $P = 0.83$; transverse: 0.51 mm, $P = 0.65$). Body-weight category remained strongly associated with splenic height in both models (both $P < 0.001$). When continuous body weight replaced weight category, the male-minus-female difference was –2.20 mm (95% CI –4.03 to –0.38, $P = 0.019$) for sagittal and –1.70 mm (95% CI –3.76 to 0.37,

P = 0.104) for transverse height. Model residuals showed no evidence of non-normality (Shapiro–Wilk, all P > 0.5). These adjusted analyses were exploratory and were not corrected for multiplicity.

Table 4. Distribution of body weight, sex, and reproductive status by body-weight category.

Weight category	N	Body weight (kg) Mean $\pm$ SD (range)	Female / Male	Intact / Neutered-spayed
<10 kg	16	5.85 $\pm$ 1.72 (3.6–8.6)	11 / 5	10 / 6
10–20 kg	14	12.51 $\pm$ 1.49 (10.5–14.9)	6 / 8	4 / 10
>20 kg	12	30.25 $\pm$ 8.25 (21.0–45.0)	6 / 6	9 / 3
Total	42	15.04 $\pm$ 11.09 (3.6–45.0)	23 / 19	23 / 19

N: number of dogs; *SD*: standard deviation. Sex and reproductive status were unevenly distributed across weight categories (females were over-represented in the <10 kg group). This uneven distribution motivated the exploratory covariate-adjusted models presented in Table 5.

Table 5. Exploratory linear models of splenic height (mm) adjusted for body weight, sex, and reproductive status.

A. Sagittal splenic height

Term	Estimate (mm)	95% CI	P	Interpretation
Model A: weight category + sex + reproductive status				
10–20 kg vs <10 kg	4.67	2.68 to 6.67	<0.001	Significantly higher
>20 kg vs <10 kg	9.51	7.63 to 11.39	<0.001	Significantly higher
Male vs female	−2.99	−4.71 to −1.27	0.001	Lower in males*
Neutered/spayed vs intact	0.20	−1.62 to 2.01	0.83	Not significant
Model B: continuous body weight + sex + reproductive status				
Per 1 kg body weight	0.328	0.250 to 0.406	<0.001	Positive linear association
Male vs female	−2.20	−4.03 to −0.38	0.019	Lower in males*
Neutered/spayed vs intact	0.49	−1.34 to 2.31	0.59	Not significant

B. Transverse splenic height

Term	Estimate (mm)	95% CI	P	Interpretation
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Model A: weight**category + sex +****reproductive status**

10–20 kg vs <10 kg	3.94	1.45 to 6.44	0.003	Significantly higher
>20 kg vs <10 kg	9.30	6.94 to 11.65	<0.001	Significantly higher
Male vs female	–2.25	–4.41 to –0.10	0.041	Lower in males*
Neutered/spayed vs intact	0.51	–1.77 to 2.78	0.65	Not significant

Model B:**continuous body****weight + sex +****reproductive status**

Per 1 kg body weight	0.332	0.244 to 0.420	<0.001	Positive linear association
Male vs female	–1.70	–3.76 to 0.37	0.10	Not significant after adjustment
Neutered/spayed vs intact	0.52	–1.55 to 2.58	0.62	Not significant

Ordinary least-squares linear regression. Model A includes body-weight category, sex, and reproductive status entered simultaneously. Model B replaces body-weight category with continuous body weight (kg). Estimates are adjusted mean differences (or the slope per kilogram of body weight). Reference categories: <10 kg, female, intact. *The lower values in males after adjustment for body weight constitute a hypothesis-generating finding; it was not hypothesized a priori and was not corrected for multiplicity. All analyses are exploratory. Model residuals showed no evidence of non-normality (Shapiro–Wilk, all $P > 0.5$).

Measurement Reproducibility:

Single-measure intra- ICC estimates based on three repeated caliper placements were 0.986 (95% bootstrap CI, 0.973–0.993) for sagittal and 0.986 (0.970–0.994) for transverse measurements for Observer 1; 0.995 (0.990–0.998) and 0.996 (0.993–0.997), respectively, for Observer 2; and 0.994 (0.989–0.997) and 0.990 (0.981–0.996), respectively, for Observer 3. Inter-observer ICC estimates, calculated using each observer’s mean of three measurements, were 0.990 (95% bootstrap CI, 0.979–0.996) for sagittal height and 0.988 (0.977–0.994) for transverse.

ICC estimates were calculated using a two-way random-effects model with absolute agreement (single measures). Bootstrap 95% confidence intervals were based on 5,000 resamples of dogs. Observers 2 and 3 independently placed calipers on the same stored images; they did not acquire new images or reposition the probe. SEM estimates and their bootstrap 95% CIs are provided in Supplementary Table S1; intra-observer SEMs refer to a single caliper placement, whereas inter-observer SEMs were based on each observer’s mean of three measurements. Inter-observer ICCs were computed as ICC(2,1) applied to each observer’s mean of three measurements.

Supplementary Table S1. Standard errors of measurement (SEM) for intra-observer repeatability and inter-observer reliability.

Reliability component	Measurement	SEM (mm)	95% bootstrap CI (mm)
Intra-observer: Observer 1 (single caliper placement)	SSpH	0.536	0.411–0.662
	TSpH	0.567	0.363–0.777
Intra-observer: Observer 2 (single caliper placement)	SSpH	0.312	0.203–0.439
	TSpH	0.310	0.246–0.369
	SSpH	0.339	0.273–0.398

Intra-observer:	TSpH	0.486	0.277–0.694
Observer 3			
(single caliper placement)			
Inter-observer	SSpH	0.451	0.300–0.593
(mean of three measurements)	TSpH	0.532	0.337–0.701

SEM was estimated from the variance components of a two-way random-effects, absolute-agreement model. Bootstrap confidence intervals were based on 5,000 resamples of dogs. Intra-observer SEMs refer to a single caliper placement; inter-observer SEMs were calculated using each observer's mean of three measurements. These SEM estimates describe measurement repeatability/reliability and are not clinical reference limits. SSpH: sagittal splenic height; TSpH: transverse splenic height.

Discussion

The size of the normal dog spleen is highly variable and must be assessed subjectively, similar to radiographic methods. However, objective sonographic data remain limited in veterinary medicine, with few systematic studies evaluating splenic dimensions across broad weight categories in clinically healthy dogs. Most of the available research has focused on cats due to the relatively narrow body weight range, and no established reference ranges currently exist for dogs [12, 13].

Against this background, the present study provides preliminary descriptive data on sagittal and transverse splenic height in clinically healthy dogs across body-weight categories. In this study when stratified by body weight, mean sagittal height increased from 11.56 ± 2.22 mm in dogs weighing <10 kg to 15.53 ± 2.06 mm in the 10–20 kg group and 20.49 ± 4.01 mm in dogs weighing >20 kg. Corresponding transverse heights were 12.27 ± 3.00 mm, 15.80 ± 2.75 mm, and 21.08 ± 3.84 mm, respectively.

Reese et al. (2013) evaluated the normal sonographic size and appearance of the spleen in 60 healthy blood-donor cats and investigated the effects of sevoflurane anesthesia and blood

collection. Splenic size was assessed in the transverse plane at the level of the splenic hilum, identified by the hilar vessels, and splenic height and width were measured perpendicular to each other using images obtained at the level where the hilar vessels were visible and maximal splenic dimensions were observed. In the present study, transverse splenic height was similarly measured at the level of the splenic hilum in the transverse plane. Splenic width was not assessed because the entire spleen could not consistently be included in a single sonographic image, particularly in larger dogs [12].

Sayre and Spaulding (2014) described the sonographic appearance and size of the spleen in 31 clinically healthy, non-sedated cats and proposed a standardized protocol for splenic evaluation. Splenic height was measured at three anatomical sites, with three measurements obtained at each site and averaged for analysis. For the splenic body, height was measured in the longitudinal (sagittal) plane as the perpendicular distance between the mesenteric and antimesenteric surfaces. This measurement approach is methodologically similar to the sagittal splenic height measurement used in the present study [13].

Auger et al. (2019) investigated the effects of commonly used sedative drugs on sonographic and radiographic splenic measurements in 15 healthy adult cats using a prospective, randomized crossover study. In their ultrasonographic protocol, splenic height was measured at the head, body, and tail in the transverse plane, with an additional measurement obtained across the splenic body in the longitudinal plane. Splenic height was defined as the perpendicular distance between the mesenteric and antimesenteric surfaces. The measurements obtained at the splenic body in both planes were methodologically similar to those performed in the present study [14].

Studies in dogs have focused primarily on the effects of anesthetics on splenic size; acepromazine and thiopental cause measurable splenomegaly, whereas propofol does not cause significant enlargement [7]. Ketamine-diazepam induction of anesthesia causes the greatest spleen volume [8]. These findings highlight the importance of standardizing anesthetic conditions; in the present study, all measurements were performed in unsedated dogs. This approach also avoids a potential source of variation associated with sedation, which has been specifically investigated in previous ultrasonographic studies of the spleen [7, 8].

The present study provides preliminary descriptive measurements of splenic height in clinically healthy dogs rather than validated clinical reference intervals. The overall mean sagittal splenic height was 15.43 ± 4.56 mm (range, 7.97–26.10 mm), and the mean transverse height was 15.96 ± 4.76 mm (range, 8.33–26.37 mm). The 95% confidence intervals reported around the means quantify uncertainty in the estimated means; they do not represent intervals expected to contain individual healthy dogs. Splenic height was defined as the mesenteric-to-antimesenteric distance measured perpendicular to the long axis at the splenic hilum in both planes. Although the ACVR and ECVDI consensus statement recommends assessing splenic thickness at the level of the hilum in a longitudinal plane—a dimension conceptually analogous to the sagittal height evaluated in this study—it utilizes a slightly different measurement protocol [15]. The mean transverse-minus-sagittal difference was 0.53 mm (95% CI, 0.01–1.04 mm), with transverse measurements slightly larger.

Splenic-height measurements differed among the three study-specific body-weight categories in both planes. Continuous body weight was strongly correlated with splenic height (Spearman $\rho = 0.83$ and 0.79 for sagittal and transverse height, respectively). Unadjusted comparisons showed no significant differences by sex or reproductive status. However, because females were over-represented among the smallest dogs, these comparisons were confounded by body weight; in exploratory models adjusted for body-weight category, males had lower splenic height than females, whereas reproductive status remained non-significant. This sex-related finding was not hypothesized a priori, was not corrected for multiplicity, rests on small subgroups (e.g., only five males in the < 10 kg category), and was less consistent when continuous body weight was evaluated (transverse plane, $P = 0.104$); it should therefore be interpreted strictly as hypothesis-generating. Furthermore, the modest sample size limits the statistical power to definitively detect or exclude other subtle subgroup associations, underscoring the need for confirmation in larger, balanced cohorts.

The values observed in the present study were numerically similar to the splenic-body heights reported by Oliveira et al. In that study, mean body height was 1.25 ± 0.21 cm in small dogs (mean weight 4.60 kg), 1.54 ± 0.31 cm in medium dogs (mean weight 10.68 kg), and 2.18 ± 0.54 cm in large dogs (mean weight 24.22 kg). The numerical similarity, particularly in medium- and large-

sized dogs, is descriptive only because the studies used different populations and measurement definitions. Both studies reported larger splenic measurements in heavier dogs.

Oliveira et al. further described the splenic body as a region with substantial thickness and volumetric capacity. In this context, the use of the splenic hilum as the measurement landmark in the present study provides a consistent location within the splenic body. However, because the two studies used different study populations and measurement definitions, the findings do not establish equivalence or interchangeability [16].

Dogs weighing >20 kg had a mean sagittal height of 20.49 mm and a mean transverse height of 21.08 mm, approximately 77% and 72% higher, respectively, than the corresponding means in dogs weighing <10 kg. This pattern may be physiologically plausible given differences in body size and circulating blood volume, but the present study did not investigate the mechanisms underlying these differences; such explanations should therefore be regarded as hypotheses.

A previous study reported mean spleen length of 6.87 ± 0.03 cm and width of 5.95 ± 0.07 cm in puppies (mean weight approximately 3 kg), compared with 4.53 ± 0.02 cm and 4.55 ± 0.04 cm, respectively, in kittens (mean weight approximately 2 kg) [17]. That study included 15 healthy male puppies and 15 healthy male kittens, all of mixed breed with a mean age of six months. Because complete visualization of the spleen in a single image was not always possible, splenic width was calculated from separate regional measurements. The measurements and populations differ from those in the present study, so the reported values are not directly comparable. The larger dimensions in puppies may partly reflect their greater mean body weight, but that study did not specifically test the relationship between body weight and splenic dimensions. Its inclusion of male animals only also limits generalization to females.

Breed was not among the primary objectives of the study, and individual breed data were not systematically recorded; breed- and conformation-related effects therefore could not be evaluated. Unadjusted comparisons by sex and reproductive status are confounded by body weight because these factors were unevenly distributed across the weight categories; the adjusted models were exploratory, were not corrected for multiplicity, and are limited by small subgroup sizes. Multiple comparisons were made in a small exploratory sample, and borderline P values (e.g., $P = 0.045$) should be interpreted cautiously. The three measurements of each observer were obtained on the

same day and only one image per plane was acquired for each dog; the reported ICC values therefore reflect caliper-placement repeatability and agreement on stored images and do not include variability due to image acquisition, and respiratory phase and probe pressure were not standardized. Establishing diagnostic thresholds for splenomegaly would require studies that include dogs with confirmed splenic disease. Body weight was categorized using study-specific cutoffs; continuous body weight was examined only by unadjusted rank correlation and exploratory linear models.

Several limitations should be considered. The sample size of 42 dogs may not capture the full variability across breeds and body conformations and is insufficient to establish formal reference intervals. Most dogs were mixed-breed, and breed representation did not support reliable breed-specific analyses; no deep-chested dogs were included. Although individual ages were available, age was not included as a covariate in the statistical analyses; therefore, a potential association between age and splenic height could not be evaluated. Furthermore, the evaluation of additional splenic dimensions, such as width, and the assessment of regional variations across the head, body, and tail of the spleen could have provided a more comprehensive characterization of splenomegaly. The study was exploratory, and no a priori sample-size calculation was performed. Future studies involving larger and more diverse populations are needed to validate these preliminary reference values and establish diagnostic thresholds in dogs with various splenic diseases, as well as to assess their clinical utility.

In conclusion, this study provides preliminary descriptive data on sagittal and transverse splenic height measured at the hilum in 42 clinically healthy dogs. Mean sagittal and transverse heights were 15.43 ± 4.56 mm and 15.96 ± 4.76 mm, respectively. Measurements differed among the three study-specific body-weight categories, whereas unadjusted comparisons showed no significant differences by sex or reproductive status; an exploratory analysis adjusted for body-weight category suggested lower values in males, which requires confirmation. These findings do not establish validated reference intervals or diagnostic thresholds. Larger studies with more diverse breed and body-conformation representation are needed.

Materials and Methods

Study Animals and Ethical Approval

Forty-two clinically healthy dogs of different breeds, ages, and body weights were included. The population consisted predominantly of mixed-breed dogs, including Pomeranian mixed-breed and Shih Tzu mixed-breed dogs in the <10 kg group, Terrier mixed-breed and Spitz mixed-breed dogs in the 10–20 kg group, and predominantly local mixed-breed and other large mixed-breed dogs in the >20 kg group. No deep-chested dogs were included. Dogs were recruited from several veterinary clinics for routine check-ups and blood testing. Eligibility required a normal clinical examination, adequate hydration, hematological and serum biochemical results within the laboratory reference intervals, and complete abdominal ultrasonography showing no abnormalities in the evaluated organs. Hematological evaluation included packed cell volume (PCV), hemoglobin, red blood cell count and indices (MCV, MCH, and MCHC), red cell distribution width (RDW), total white blood cell count, leukocyte differential count, and platelet count. The serum biochemical panel included total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, glucose, total cholesterol, triglycerides, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium, and phosphorus. Owners were asked about the dogs' health, medications, vaccination, reproductive status, pregnancy, and lactation; dogs that had received medication or vaccination, or had been pregnant or lactating, within the preceding six months were excluded. For descriptive comparisons, dogs were classified into study-specific body-weight categories (<10 kg, 10–20 kg, and >20 kg).

The study was reviewed and endorsed by the Research Ethics Committee of Islamic Azad University, Science and Research Branch, Tehran, Iran.

All procedures were performed in the presence of the owners and were completed within approximately one hour. No sedation or anesthesia was administered; examinations were performed using physical restraint only. After the evaluations were completed, the dogs were returned to their owners.

Splenic Imaging and Measurements

Dogs were fasted for 8–12 hours prior to examination and gently restrained in dorsal recumbency without receiving any sedatives or anesthetics. The spleen was evaluated ultrasonographically in two perpendicular planes (sagittal and transverse) using a 3–12 MHz multi-frequency linear-array transducer with an ALPINION E-CUBE i7 ultrasound system (Alpinion Medical Systems, Seoul,

South Korea). Operating frequency, depth, gain, and focal zone were customized for each individual dog to achieve optimal image quality. No specific standardization of respiratory phase or probe pressure was applied beyond gentle contact sufficient to obtain a clear image. All splenic measurements were obtained at the level of the splenic hilum; images were acquired only when the hilar vessels were clearly visible. In the sagittal plane, splenic height was measured as the maximum distance between the mesenteric and antimesenteric surfaces, with the measurement line oriented perpendicular to the long axis of the spleen (Figure 1). In the transverse plane, the corresponding mesenteric-to-antimesenteric dimension was measured in the same manner and recorded as transverse splenic height (Figure 2). The initial ultrasonographic examinations and all primary measurements were performed by one primary observer (Observer 1), who obtained three repeated measurements for each plane, on the same stored standard image. The mean of these three measurements was used for all subsequent statistical analyses of splenic dimensions, body-weight groups, sex, and reproductive status.

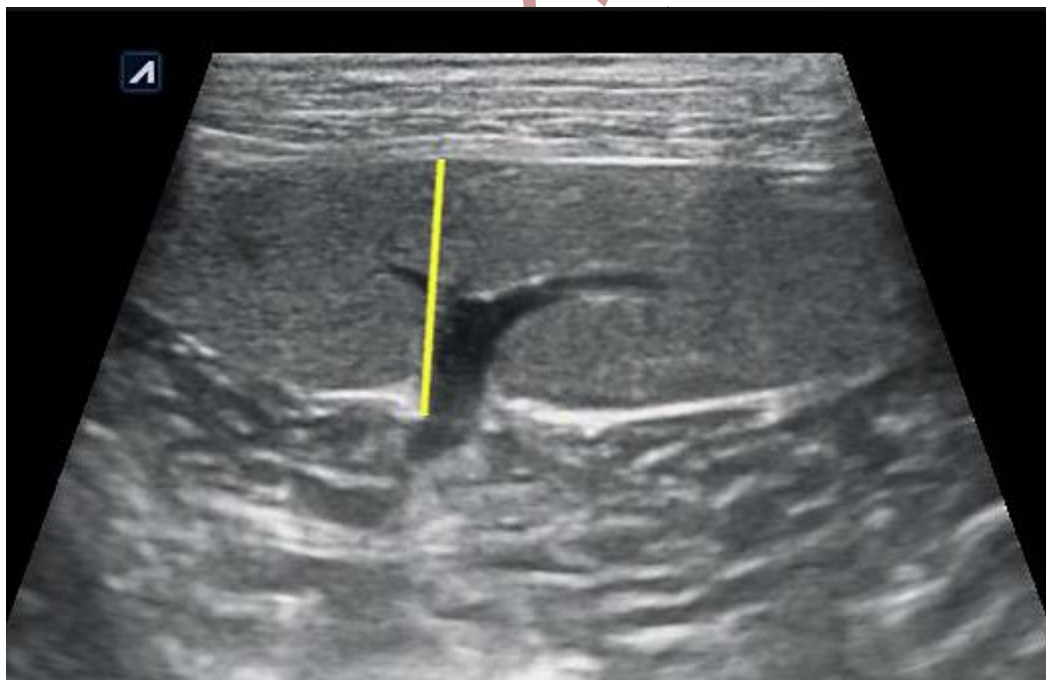


Figure 1. Ultrasonographic measurement of sagittal splenic height at the level of the splenic hilum in a clinically healthy dog. Splenic height is defined as the distance between the mesenteric and antimesenteric surfaces, measured perpendicular to the longitudinal axis of the spleen in the sagittal plane. Measurements are expressed in millimeters (mm).

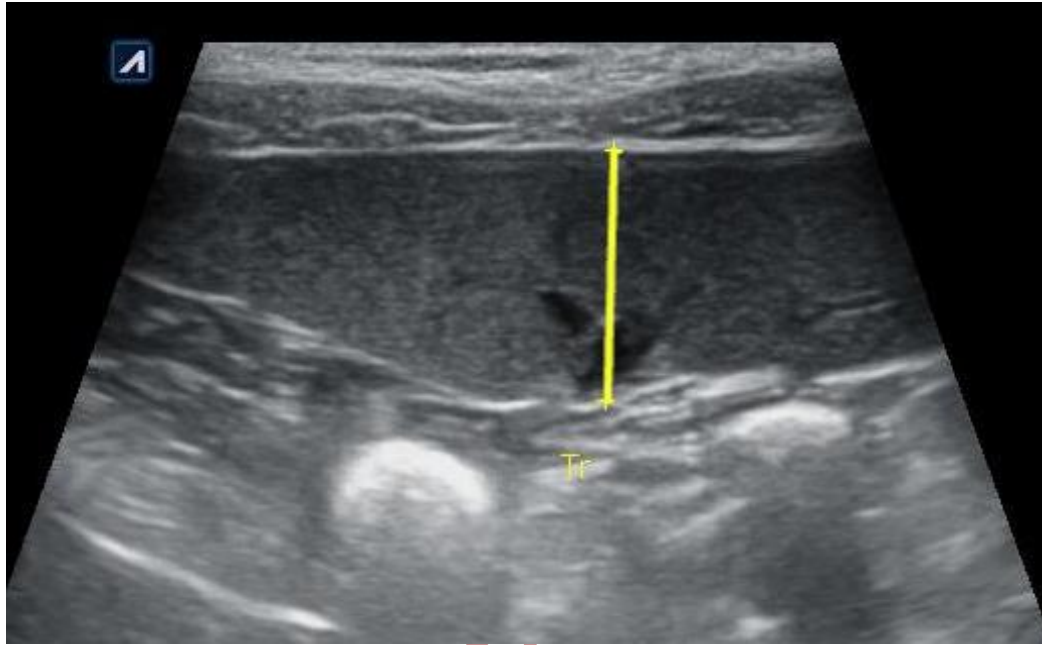


Figure 2. Ultrasonographic measurement of transverse splenic height at the level of the splenic hilum in a clinically healthy dog. Splenic height is defined as the distance between the mesenteric and antimesenteric surfaces, measured perpendicular to the longitudinal axis of the spleen in the transverse plane. Measurements are expressed in millimeters (mm).

All three observers were veterinarians with more than 7 years of professional experience. Observer 1 performed the ultrasonographic examinations and obtained all measurements after image acquisition, whereas Observers 2 and 3 performed their measurements retrospectively on the recorded images. Subsequently, two additional observers (Observers 2 and 3) independently placed calipers on the same stored ultrasonographic images on three occasions to assess intra-observer repeatability and inter-observer reliability. These were repeat measurements of stored images, not separate image acquisitions. All observers were blinded to previously obtained measurement values and to the results of the other observers. For all observers, the three measurements were obtained on the same day, using image-analysis software that did not display previous measurement values or caliper placements.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA), except Dunn pairwise comparisons with Holm adjustment, effect sizes,

bootstrap confidence intervals, Mann–Whitney sensitivity tests, covariate-adjusted linear models, and Spearman correlations, which were computed in Python 3 (SciPy and NumPy). For each dog and plane, the mean of Observer 1’s three repeated measurements was used for all primary clinical analyses. Descriptive statistics included the mean, standard deviation (SD), range, standard error of the mean (SEM), and 95% confidence intervals (CIs) for means. Normality was assessed using the Shapiro–Wilk test, including the distribution of within-dog paired differences for the comparison between measurement planes. Because the paired differences showed no evidence of non-normality, the paired t-test was used as the primary comparison between sagittal and transverse heights, with the Wilcoxon signed-rank test retained as a sensitivity analysis. Comparisons by sex and reproductive status were performed using Welch’s independent-samples t-tests, which do not assume equal group variances; these exploratory comparisons were interpreted cautiously. As sensitivity analyses, these comparisons were repeated using the Mann–Whitney U test. To address confounding by body weight, sagittal and transverse heights were additionally modelled by ordinary least-squares linear regression with body-weight category (or, in a second model, continuous body weight), sex, and reproductive status entered simultaneously; residual normality was checked with the Shapiro–Wilk test, and these adjusted models were exploratory, with no correction for multiplicity. Associations between continuous body weight and splenic height were assessed using Spearman’s rank correlation. Differences among body-weight categories were assessed using the Kruskal–Wallis test followed by Dunn pairwise comparisons with Holm adjustment. The Kruskal–Wallis effect size was calculated as $\eta^2_H = (H - k + 1)/(n - k)$, where H is the Kruskal–Wallis statistic, k is the number of groups, and n is the total sample size. A P value <0.05 was considered statistically significant. Intra-observer repeatability and inter-observer reliability were assessed using intraclass correlation coefficients (ICCs) with a two-way random-effects, absolute-agreement, single-measure model; bootstrap 95% CIs were based on 5,000 resamples of dogs. Inter-observer ICCs were computed as ICC(2,1) applied to each observer’s mean-of three measurements. Standard errors of measurement (SEMs) were estimated from the variance components of the same absolute-agreement model, with bootstrap 95% CIs based on 5,000 resamples of dogs.

Author Contributions:

Mahshid Ghavidel: Conceptualization, Data collection, Ultrasonographic examinations, Writing – original draft.

Farrokhreza Kabir: Study design, Supervision, Manuscript review and editing.

Veria Towhidi: Conceptualization, Manuscript review.

Bitā Vazir: Conceptualization, Manuscript review.

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Conflict of Interest

The authors declare no conflicts of interest.

Ethical Approval and Consent to Participate

The study was reviewed and endorsed by the Research Ethics Committee of Islamic Azad University, Science and Research Branch, Tehran, Iran. Written informed consent was obtained from all animal owners.

Data Availability Statement

The raw ultrasonographic measurements and datasets generated during the current study are available from the corresponding author upon reasonable request.

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بررسی سونوگرافیک ارتفاع طحال در سگ‌های سالم از نظر بالینی در گروه‌های مختلف وزنی

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نویسنده مسئول: دکتر فرخ‌رضا کبیر

چکیده

ارزیابی دقیق ابعاد طحال با سونوگرافی در بررسی سگ‌های مشکوک به اسپلنومگالی اهمیت دارد، اما داده‌های استاندارد سگ‌ها که تغییرات وزن بدن را لحاظ کنند، همچنان محدود هستند. این مطالعه، ارتفاع سونوگرافیک طحال را در سگ‌های از نظر بالینی سالم مشخص کرده و تفاوت‌های آن را بر اساس گروه‌های وزنی، جنسیت و وضعیت تولیدمثلی ارزیابی نمود. چهل و دو سگ سالم از نظر بالینی تحت معاینه فیزیکی، غربالگری هماتولوژیک و بیوشیمیایی سرم، و سونوگرافی کامل شکم قرار گرفتند. ارتفاع‌های ساژیتال و ترانسورس طحال در سطح ناف طحال اندازه‌گیری شدند. سگ‌ها در دسته‌های وزنی اختصاصی مطالعه (>10 کیلوگرم، $10-20$ کیلوگرم و <20 کیلوگرم) طبقه‌بندی شدند. میانگین $\pm$ انحراف معیار ارتفاع‌های ساژیتال و عرضی به ترتیب $4/56 \pm 15/43$ و $4/76 \pm 15/96$ میلی‌متر بود. میانگین اختلاف بین اندازه‌گیری‌های ارتفاع عرضی و ساژیتال $0/53$ میلی‌متر (فاصله اطمینان 95% : $0/01$ تا $1/04$ میلی‌متر؛ آزمون t زوجی، $P=0/045$) بود و مقادیر ارتفاع در نمای عرضی به طور سیستماتیک بیشتر بودند؛ آزمون رتبه علامت‌دار ویلکاکسون مقدار $P=0/018$ را نشان داد. ارتفاع طحال در هر دو صفحه در سه دسته وزن بدن متفاوت بود (برای هر دو $P<0/001$). هیچ تفاوت آماری معنی‌داری بدون تعدیل بر اساس جنسیت یا وضعیت تولیدمثلی شناسایی نشد؛ در یک مدل اکتشافی تعدیل‌شده بر اساس دسته وزن بدن، ارتفاع‌های ساژیتال و عرضی در نرها کمتر از ماده‌ها بود (به ترتیب $2/99$ میلی‌متر، $P=0/001$ ؛ و $2/25$ میلی‌متر، $P=0/041$)، در حالی که وضعیت تولیدمثلی همچنان غیرمعنی‌دار باقی ماند. وزن پیوسته بدن همبستگی قوی با ارتفاع نشان داد (ضریب همبستگی اسپیرمن $0/83$ و $P=0/79$) این یافته‌ها داده‌های توصیفی مقدماتی را فراهم می‌کنند و به دلیل حجم نمونه متوسط و تنوع نژادی محدود، نیازمند تفسیر محتاطانه و ارزیابی بیشتر در جمعیت‌های بزرگ‌تر است.

کلیدواژه‌ها: طحال سگ؛ اولتراسونوگرافی؛ ارتفاع طحال؛ وزن بدن