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Research Article

Ultrasonographic Assessment of Liver Size and its Association to Selected Morphometric Parameters of Domestic Dogs in Accra, Ghana

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ABSTRACT

Introduction: The global unpopularity of linear ultrasonographic measurement, due to its inherent subjectivity, contrasts with the safety, portability, low cost, and real-time capabilities of this imaging modality. The increased availability of ultrasounds in veterinary practice in Ghana presents an opportunity to provide ultrasonographic liver size reference ranges to aid the diagnosis of hepatopathies in domestic dogs. Therefore, this study sought to establish ultrasonographic liver size reference ranges of dogs in Accra, Ghana. It also aimed to investigate the correlation between liver size and selected morphometric parameters in these domestic dogs.
Materials and methods: A total of 60 dogs from different domestic breeds, sexes (27 males and 33 females), age ranges (2.82 ± 2.12 years), weights (28.83 ±9.98kg), and body conformation were sampled. Purposive sampling of dogs was performed based on presenting history, clinical signs, physical exam, and blood analysis. Blood samples were collected for serum biochemistry to distinguish between those classified as healthy and those presenting with clinical illness. Additionally, all dogs were subjected to linear ultrasonographic liver size measurements in longitudinal and transverse planes.
Results: The findings indicated a strong positive correlation of mean longitudinal sonographic liver measurement with body height, body girth (the widest point of the chest and the rib cage), the distance between the last rib and the tuber coxa, and the distance between the xiphoid and the tuber ischium. Equations were derived from the mean longitudinal sonographic measurement and these body parameters for deep and non-deep-chested breeds. This study helped to establish equations that can be used to estimate the longitudinal liver measurement.
Conclusion: This information can be used in clinical settings to help veterinarians (even with basic knowledge of hepatic ultrasonography) to have a fair idea of hepatopathies relating to size.

1. Introduction

Ultrasound is a non-invasive, effective, inexpensive, and comparatively safer modality compared to other imaging modalities, including X-rays and computed tomography (CT) for detecting pathologies[1-4]. Using this modality in the veterinary hospital setting has been proven to improve animal physical examination accuracy[5,6]. Liver ultrasonography in animals, in particular, has proven to be an indispensable tool and is indicated for clinical signs,

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such as icterus, vomiting, and anorexia exhibited by a patient. The suspicion of certain hepatopathies and alterations on a serum liver function test (LFT) may also warrant this examination [1-3,7,8].

Among other factors, knowledge of liver anatomy[9] is necessary for accurate diagnoses or interpretation of liver ultrasonography[10]. The identification of acute and chronic liver ultrasonographic lesions is depicted by the hepatic size, shape, echo pattern, and texture[11].

However, findings from previous ultrasonographic studies appear incongruent with the determination of hepatic size measurements[8,12,13]. The use of liver size measurements from canine hepatic ultrasonography, dependent on subjectivity, has made a typically safe, accessible, inexpensive, and real-time imaging method contentious. This is attributed to the unavailability of reliable reference ranges, whether generalized, species-specific, or body conformation-specific.

This study aimed to establish a generally accepted reference range for ultrasonographic liver size measurement in dogs regardless of breed influences. The study also sought to compare the diagnostic accuracy of liver ultrasonography against serum liver function tests in determining hepatopathies and to develop a model for predicting standard liver size based on selected morphometric features.

2. Materials and Methods

2.1. Study design and population

This cross-sectional study, conducted from January 2022 to July 2022, involved a sample of 60 dogs representing various breeds, including Doberman, German Shepherd Dog, Boer Boel, Local dog (Avuvi/mongrel), and Rottweiler. These breeds represent the most commonly presented dogs at the Small Animal Teaching Hospital (SATH), University of Ghana, where the study was carried out. The selection criteria prioritized dogs that appeared healthy. To be included in the study, dogs were required to indicate normal vital signs, including a normal temperature (38.5-39.4°C), normal pulse (80-160 bpm with reference ranges as per SATH), and no signs of illness, such as edema, ascites, icterus, alopecia, and visible skin masses. They were neither candidates for any form of surgery nor undergoing any form of medical treatment at the time of presentation. Hence, dogs presented for routine medical procedures, such as regular check-ups, vaccinations, deworming, and grooming, were preferred. Participants whose liver function test results showed no signs of damage or malfunction were categorized as apparently healthy, while those with test results indicating liver damage or malfunction were classified as clinically ill. Subsequently, all participants underwent ultrasonography for further evaluation.

2.2. Study area

The study was conducted at SATH, University of Ghana School of Veterinary Medicine. The teaching facility operates Mondays to Saturdays (8:00 am to 5:00 pm) and receives an average of 80 patients weekly. The facility also provides both ultrasound and laboratory services and can identify and analyze liver biomarkers, including Alanine Transferase (ALT), Aspartate Transferase (AST), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), Bilirubin, Total proteins, and Albumins. SATH allows for safe and ethically approved patient research as part of its mandates. This made the hospital a viable location for this study.

2.3. Sample size and sampling method

A total of 60 dogs were used for the study. The participants were recruited using a stratified proportionate probability sampling technique[14]. The two strata were apparently healthy dogs and clinically ill dogs. Each stratum constituted 30 dogs irrespective of sex. The clinically ill dogs were recruited to control the study as the diseases presented by these clinically ill dogs could cause multi-organ dysfunction affecting internal organs such as the liver, spleen, and kidneys[3].

2.4. Inclusion and exclusion criteria

On arrival, dogs belonging to the breeds of interest underwent a thorough physical examination. This was before obtaining a clinical history to categorize the dogs as healthy or clinically ill. Healthy dogs were presented for routine exams, vaccinations, and worming with unremarkable physical examinations and LFT results. Clinically ill dogs presented with remarkable physical examination and LFT results.

2.5. Laboratory reclassification and liver function tests

To begin, 3 ml of blood from the cephalic vein was taken using a sterile 5 mL syringe and dispensed into a serum separator tube. The sample was instantly sent to SATH's diagnostic laboratory for analysis using a fully automated veterinary chemistry analyzer (URIT® 8021A, China). The parameters assessed included AST, ALT, GGT, and ALP. The LFT, therefore, served as a measure to select a 'truly' apparently healthy animal for the study to obtain normal ranges for liver size measurements. Reference intervals established by the Small Animal Teaching Hospital, University of Ghana (in use at the hospital but unpublished) were used as recommended by Barsanti[15]. The ranges were sourced initially from that provided by the Clinical Pathology Laboratory at Cornell University[16] and eventually tailored for the conditions in Ghana.

2.6. Data collection

A self-designed questionnaire and an ultrasound machine (American Megatrends®, India) with a 4 MHz curvilinear probe were used for data collection. Data on body girth, body height, the measured distance between the last rib and the tuber coxa, the measured distance between the xiphisternum and tuber ischium, and longitudinal and transverse ultrasound liver measurements were recorded as continuous variables while data on sex, sexual status, breed, and body conformation were recorded as categorical variables.

2.7. Ultrasonographic measurements

The area of hair from the xiphisternum to the umbilicus caudally and along the lateral borders of the last ribs was clipped as described by Barr[17] and Mauragis and Berry[18], using Oster® clippers, USA. This ensured that the ultrasound probe made clean contact with the skin during ultrasound measurements of the liver size. The skin was then cleaned with surgical spirit, and the patient was placed on dorsal recumbency. A liberal amount of acoustic gel was applied, and the patient rested for 2 minutes before the scan. As Thrall[13] and Mannion[19] explained, the acoustic gel and the displacement of air between the skin and the probe reduce the formation of artifacts during the ultrasound scan. No anesthetics were used[18] as patients were physically restrained. A 4MHz curvilinear probe was used to scan each patient in longitudinal and transverse planes. For the longitudinal plane, the probe was placed perpendicularly on the midline caudal to the xiphisternum, and a clear image was obtained on the monitor which was frozen at maximum expiration[19]. A clear image was required prior to freezing and subsequent measurements of the liver in the longitudinal and transverse planes. This was necessary as the dogs used for the study were manually restrained, resulting in various movements on the examination table. A clear image showed the caudal liver surface as near vertical as possible while retaining a clear image of the diaphragm line and the liver parenchyma[18]. Freezing the image at maximum expiration ensured a decrease in the air trapped between the abdominal wall and the liver, reducing the odds of artifact formation as much as possible while enhancing the image obtained. A two-line length of the liver was taken from the greater curvature of the liver (on the right side of the screen) diagonally to the skin surface (on the left of the screen), measured, and recorded (mm). The procedure was repeated three times, and each value was registered as a unique figure with the prefix L followed by a number, for example, L1 and L2. For the transverse plane, the probe was first placed on the long-axis plane at the xiphisternum and then rotated 90 degrees towards the sonographer. The image was frozen once a clear view was obtained. The distance from the dorsal border of the liver was measured (mm) to the ventral tip and recorded as the transverse plane hepatic size measurement. The measurement was repeated three times, and each value was registered as a unique figure with the prefix T followed by the corresponding number, for instance, T1 and T2 (Figures 1, 2).

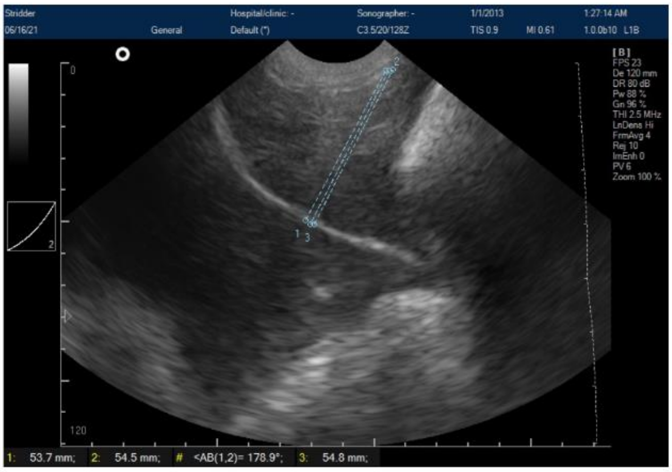

Figure 1. Longitudinal sonogram of the liver measurement of a dog

The probe was placed 90 degrees from the midline caudal to the xiphisternum, and the image was frozen at maximum expiration. A two-line length from the greater curvature of the liver diagonally (blue lines) to the skin surface was then measured (Figure 1).

The probe, first placed on the long axis plane at the xiphisternum, was rotated 90 degrees towards the sonographer, and the image was frozen. The distance from the dorsal border to the ventral tip (blue lines) of the liver was measured (Figure 2).

2.8. Data analysis

Statistical Package for the Social Sciences version 25.0 (SPSS, Chicago, IL, USA) was used for data analysis. For descriptive statistics, summaries of categorical variables were analyzed using frequencies and percentages, while summaries of continuous variables were reported as means with corresponding standard deviations. For inferential statistics, the p-value was considered significant at $p \leq 0.05$. The independent t-test and one-way Analysis of Variance (ANOVA) were used to check for a significant difference in the variables between apparently healthy and clinically ill participants. Pearson correlation was used to determine the degree of association between the

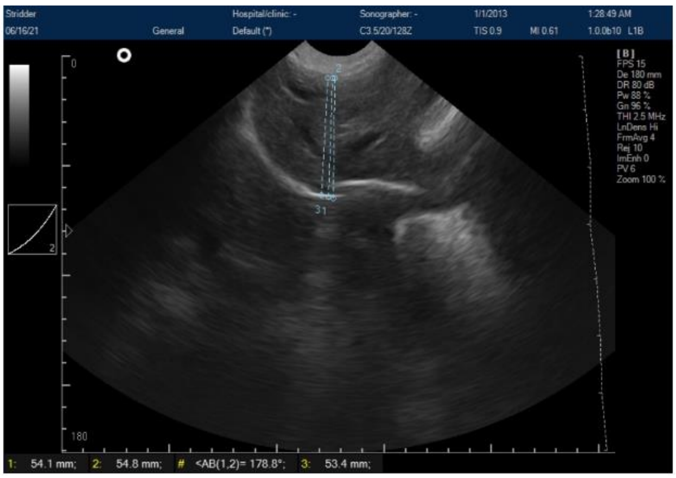

Figure 2. Transverse sonogram of the liver measurement of a dog

continuous variables. Multiple linear regression was performed to predict standard liver size in dogs based on the variables that showed significant associations.

3. Results

A total of 60 dogs were sampled, comprising 30 apparently healthy dogs and 30 clinically ill dogs. Ages ranged from 6 months to 10 years, with a mean age of 2.82 ± 2.12 years. The weights of the dogs also ranged from 10 kg to 50 kg, with a mean of 28.83 ± 9.98 kg. Other characteristics, such as sex, sexual status, breed types, and deep-chested or non-deep-chested are summarized in Table 1. Descriptive statistics of the sampled dogs, for their physical examination parameters and LFT values, are indicated in Table 2.

The independent t-test showed a statistically significant difference in the mean longitudinal liver measurement in apparently healthy deep-chested and non-deep-chested dogs ($p = 0.05$) and no significant difference in mean transverse liver measurements in apparently healthy deep-chested and non-deep-chested dogs ($p = 0.14$). Moreover, the test showed a statistically significant difference in the mean longitudinal liver size measurement ($p = 0.05$) and no statistically significant difference in the mean transverse liver measurement ($p = 0.19$). Between apparently healthy and clinically ill dogs, the analysis demonstrated a statistically significant difference in mean longitudinal liver measurement and the weights ($p < 0.001$) of apparently healthy dogs ($p < 0.001$). Pearson correlation of sonographic liver measurements and body measurements of apparently healthy dogs showed a moderate positive correlation between mean longitudinal sonographic liver measurements and body height ($r = 0.646$, $p < 0.001$), a strong positive correlation between mean longitudinal liver measurements and body girth ($r = 0.793$, $p <0.05$), a moderate positive correlation between mean longitudinal liver measurements and the distance between the xiphoid and the tuber ischium ($r = 0.45$, $p <0.05$), and a moderate positive correlation between longitudinal liver measurements and the distance between the last rib and the tuber coxa ($r = 0.638$, $p < 0.05$).

Linear regression equations predicting the mean longitudinal liver size using body weight, body girth, and distance between the last rib and the tuber coxa were used for healthy deep-chested and non-deep-chested dogs. Concerning deep-chested dogs, the longitudinal liver size (mm) can be estimated as 43.93 + 1.04 (body weight), 2.542 + 0.96 (body girth), or 18.776 + 0.332 (distance between last rib and tuber coxa). Concerning non-deep chested healthy dogs, the longitudinal liver size (mm) can be estimated as 43.93 + 1.04 (body weight), 42.808 + 0.52 (body girth), and 123.282 – 245 (distance between last rib and tuber coxa).

Ultrasonographic liver size measurements (longitudinal lengths) of the breeds used for this study were thus determined to be Avuvi/mongrel (52.1-63.9 mm), Boer Boel (54.5-92.4 mm), Doberman (71.6-86.3 mm), German

Table 1. General characteristics of dogs sampled at the Small Animal Teaching Hospital, Accra, Ghana

parameter	Characteristics	Frequency	percentage (%)
Sex	Male	27	45
	Female	33	55
Sexual status	Intact	60	100
	Neutered	0	0
Breed	Boer Boel	22	37
	Doberman	11	18.3
	German Shepherd Dog	10	16.7
	Mongrel	11	18.3
	Rottweiler	6	10.0
Body conformation	Deep chested	38	63.3
	Non-deep chested	22	36.7

Table 2. Descriptive statistics of dogs sampled at the Small Animal Teaching Hospital, Accra, Ghana

Description	Variable	Ref	Apparently Healthy			Clinically ill		
			Min[a]	Max[a]	Mean[a] ± SD	Min[c]	Max[c]	Mean[c] ± SD
Vital signs	Temperature (°C)	38.5 –39.4	38	40.7	38.96 ± 0.57	38.2	41	39.30 ± 0.63
	pulse (bpm)	80 - 140	120	160	134.77 ± 10.91	88	182	149.03 ± 0.90
Body measurement	Body height (mm)	-	420	760	595.00 ± 89.40	450	740	603.00 ± 74.75
	Body girth (mm)	-	370	890	707.00 ± 127.96	530	870	738.67 ± 85.13
	Distance between the xiphoid and Ischium (mm)	-	210	450	353.00 ± 46.84	320	520	393.33 ± 47.80
	Distance between last rib and tuber coxa(mm)	-	80	190	152.33 ± 27.88	120	210	168.00 ± 18.08
	Mean longitudinal liver measurement (L/mm)	-	33.4	98.4	72.14 ± 16.86	50.7	105.8	79.73 ± 12.23
	Mean transverse liver measurement (T/mm)	-	46.1	89.5	67.83 ± 11.22	46.9	117.6	72.47 ± 15.44
Liver enzymes	Total bilirubin (μmol/L)	0.0 – 5.1	0.4	32.5	9.38 ± 6.85	1.8	42.2	13.14 ± 7.56
	Direct bilirubin (μmol/L)	1.0 - 2.0	0.6	64.9	6.03 ± 11.56	0.8	16.6	3.58 ± 3.20
	ALT(U/L)	10 -109	11	86	30.53 ± 18.03	13	238	52.20 ± 57.32
	AST(U/L)	9 - 51	120	559	64.23 ± 100.21	26	263	124.73 ± 71.70
	ALP(U/L)	1 - 114	27	263	96.20 ± 62.82	12	350	160.53 ± 92.06
	GGT(U/L)	3 - 19	0.0	35	7.30 ± 8.15	0.0	92	12.90 ± 20.51
	Total protein (g/L)	54 - 75	17.8	79.8	57.22 ± 12.79	16.6	96.3	56.41 ± 17.14
	Albumin(g/L)	23 - 31	12	44	31.72 ± 7.76	10.4	46.3	25.27 ± 8.36

SD: Standard deviation, a: Apparently healthy, c: Clinically ill. The breeds of dogs sampled are: German Shepherd Dog, Boer Boel, Avuvi, Rottweiler, and Doberman, ALT: Alanine transaminase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma glutmintrnsaminase

Shepherd Dog (72.8-81.4 mm), Rottweiler (71.6-74.1 mm). In apparently healthy dogs, one-way ANOVA tests between mean sonographic liver measurements and body condition score (BCS) in apparently healthy dogs showed p = 0.18 and p = 0.28 for mean longitudinal liver measurement and body condition score, as well as mean transverse liver measurement and BCS, respectively. A similar test showed p values of 0.05 and 0.45 for mean longitudinal liver measurement and age and mean transverse liver measurement and age, respectively.

4. Discussion

A total of 60 dogs were recruited, 30 being apparently healthy and the remaining clinically ill as a control sample. The sample size exceeded that adopted by Godshalk et al.[12] and Barr[17] who examined 16 and 50 dogs, respectively. However, the sample size was less than that reported by Barr[20,] investigating 100 dogs.

This study sample was female-dominated, with 27 (45 %) males and 33 (55 %) females. The differentiation of sexes was also observed in a study by Godshalk et al.[12], where 8 (50 %) females and 8 (50 %) males were sampled. The minor differences in the female-to-male ratio in this study could be attributed to behavioral and perceptional differences between the male and female dog populations by owners[21]. People prefer female to male dogs because of their perceived ease of handling and breeding. However, such observable differences are usually ascribed to the use of female dogs for the dog breeding business (selling of puppies) here in Ghana.

None of the previous studies considered the effect of age on the ultrasonographic liver size. The average age in this study was 2.82 ± 2.12 years. There was a statistically significant relationship (p = 0.05) between mean longitudinal liver measurement and the age of apparently healthy dogs.

Most dogs sampled were adolescents (68.3 %), with the least being adults (6.7 %). The weight of animals tested for the ultrasonographic liver measurements in previous studies was 7.7 – 29 kg and 1 – 60 kg by Godshalk et al.[12] and Barr[17,20] respectively. The weight range of all sampled dogs in this study was 10 – 50 kg (average of 28.9 kg). Given the average age and selected breeds of dogs (medium-sized) for this study, the average weight realized in this study was within the weight ranges of medium-sized dogs as identified by major internationally accepted dog clubs, including American Kernnel Club[22] and South African Boer Boel Breeders' Society[23].

It is recommended to use the reference values specific to the laboratory for deriving data for a particular sample[15]. Therefore, the results were interpreted using the reference intervals provided by the Small Animal Teaching Hospital laboratory. Animals declared healthy on average had relatively higher total bilirubin, direct bilirubin, and AST. The rest of the LFT parameters were within range. Since spikes in total and direct bilirubin and AST alone are not enough to conclude a loss in function and/or damage to the liver[24], the assessment that the animals sampled were apparently healthy and without any liver pathologies was most likely correct.

While Godshalk et al.[12] performed a complete blood count (CBC) and serum chemistry profile to evaluate hepatopathies in all dogs, it was not aimed at differentiating apparently healthy dogs from clinically ill dogs. Granulomatous hepatitis in a dog and mild alterations in eight other dogs were detected only at post-mortem[12]. This confirms the argument that CBC and LFT observations are insufficient to diagnose most hepatic alterations. Hence, a justification for the elimination of CBC as a test to determine liver health in this study.

In the current study, apparently healthy dogs showed a strong positive correlation between mean longitudinal sonographic liver measurement and body height, body girth, the distance between the last rib and the tuber coxa, a moderate positive correlation between mean longitudinal liver measurements and the distance between the xiphoid and the tuber ischium. Thus, in apparently healthy animals, these parameters would significantly increase longitudinal sonographic liver measurements in dogs. The significant association between sonographic mean longitudinal liver measurements and the weights (below 20 kg and above 20 kg) of apparently healthy dogs was also observed by Barr[20]. According to her, once the body weight has been taken into account, the liver size is independent of the sex of the Animal. Therefore, better indicators of liver sizes could be breed and age. However, given that the breed and age are factors of the dog's physique, the determinant of liver size could be based on the dog's physique.

Breeds of dogs who presented as apparently healthy showed a statistically significant difference between the mean longitudinal sonographic liver measurement and the differences in breed physique (deep-chested or non-deep-chested). Thus, the mean longitudinal sonographic liver measurement depends on whether a dog breed is deep-chested or non-deep-chested. Barr[20] also made an identical observation. Based on regression analysis on each of the body groups, she determined that the liver size (mm) of a deep-chested dog can be determined by 69.3 +1.4(body weight/Kg) and that the liver size (mm) of a standard non-deep-chested dog is determined using 56.4 + 1.5(body weight/kg). From this study, the mean longitudinal liver size (mm) of the deep-chested was 43.93 + 1.04(bodyweight/kg) while that of the non-deep-chested dog was 29.43 + 1.57 (bodyweight/kg). While the equations generated by both studies are significantly different, both appear to suggest that, for a given weight, deep-chested dogs will have a relatively larger longitudinal liver size than non-deep-chested dogs. These established ranges can serve as an additional guide in determining certain hepatopathies that significantly impact the length of the canine liver, including acute hepatic and chronic hepatic disease (CHD) conditions[11].

Regression analysis using liver-specific biomarkers to diagnose hepatic conditions accurately was not statistically significant. Thus, elevated liver biomarkers in dogs do not signify a hepatic disease condition in the clinical setting. This is especially so because isoenzymes or isoforms of these hepatic enzymes are present in other tissues, such as muscle and bone[25]. Linear regression analysis of sonographic liver and anatomical body measurements showed a significant association between body girth, body height, the distance between the last rib and the tuber coxa, and the longitudinal sonographic measurement. This association was determined for both deep-chested and non-deep-chested breeds. Equations derived can be used to estimate liver sizes in clinical settings. Using these equations can guide clinicians in deciding hepatopathies relating to changes in size when using ultrasounds.

5. Conclusion

From the study, longitudinal ultrasonographic liver size reference ranges established for apparently healthy dogs sampled in the survey are Avuvi/mongrel (52.1-63.9 mm), Boer Boel (54.5-92.4 mm), Doberman (71.6-86.3 mm), German Shepherd Dog (72.8-81.4 mm), Rottweiler (71.6-74.1 mm). The longitudinal length of the canine liver can be estimated using the following equations: Y = 29.14 + 1.57 (body weight) and X = 43.93 + 1.04 (body weight) for non-deep and deep-chested breeds, respectively. More so, measured body height and body girth can be used as a predictor of the longitudinal ultrasonographic liver size measurements in apparently healthy dogs using the following equations: V = 0.122(body height) - 0.355 and Z = 0.104(body girth) - 1.690 respectively. Further studies, using larger sample sizes need to be conducted to validate the various equations derived from this study, which will help facilitate longitudinal ultrasonographic liver size measurements of domestic canines.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed to the study design, collecting samples, laboratory tests, data analysis, and writing the draft of the manuscript. The authors checked and approved the last edition of the manuscript before publication.

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Availability of data and materials

All relevant data have been attached as supplementary. However, the corresponding authors can be contacted for any additional information.

Ethical considerations

The ethical concerns of plagiarism, permission to publish, misconduct, data fabrication and falsification, double publishing, submission, and redundancy have all been reviewed by the authors. Authors are responsible for disclosing all relationships and activities that might bias or be seen to bias their work.

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