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Papers

Ultrasonographic evaluation of the adrenal glands in healthy dogs: repeatability, reproducibility, observer-dependent variability, and the effect of bodyweight, age and sex

G. Mogenicato, C. Layssol-Lamour, F. Conchou, A. Diquelou, F. Raharison, J. Sautet, D. Concordet

Adrenal length and width were determined from two-dimensional ultrasound longitudinal images. In study 1, 540 measurements of adrenal glands were attempted from five healthy beagle dogs by three different observers with different levels of expertise in ultrasonography, to determine the variability of adrenal gland measurements. Of these, 484 measurements were included in the statistical analysis, since 16 measurements of the left adrenal gland and 40 for the right could not be visualised by the observer. In study 2, a single measurement of both adrenal glands was taken from each of 146 dogs by the most trained observer from study 1, and the effects of different health status (healthy dogs v dogs with non-adrenal diseases), bodyweight, age and sex were assessed. A total of 267 measurements were included in the statistical analysis. The lowest intra- and inter-day coefficient of variation values were observed for the left adrenal gland and by the most trained observer. The health status had no statistically significant effect on adrenal gland length or width, whereas age had a significant effect only for the left adrenal gland (the greater the age, the greater the width or length) and sex had a significant effect only for the right adrenal gland (the width was larger in males and the length larger in females). The bodyweight had a significant effect for the length of both adrenal glands (the greater the bodyweight, the greater the length), but not the width. The differences between sd and coefficient of variation values for the width of the left adrenal gland were not statistically significant between the three observers, whereas they were statistically significant for the right adrenal gland.

MULTIPLE imaging modalities, including radiography (Penninck and others 1988), CT (Voorhout 1990), scintigraphy (Munlich and others 1976) and ultrasonography (Schelling 1991), are used to assess adrenal gland size. Ultrasonography is also useful as a primary screening modality for human patients with suspected adrenal

lesions (Yeh 1988). For dogs, the clinical utility of diagnostic ultrasound for evaluating diseases of the adrenal gland has been described (Kantrowitz and others 1986, Saunders and others 1992), in particular for pituitary-dependent hyperadrenocorticism (PDH) (Schelling 1991). Indeed, several ultrasonographic studies have shown that the adrenal glands of dogs with PDH are bilaterally enlarged with a normal shape (Schelling 1991, Grooters and others 1996). Moreover, Barthez and others (1995) found, using transverse adrenal measurements, an 81 per cent sensitivity and 100 per cent specificity for differentiating normal dogs from dogs with PDH when an upper limit of 7.5 mm for the left adrenal gland width was used.

However, the relevant interpretation of these results is dependent on the performance of the measurements. All measurements can be divided into two terms: actual value and noise. Evaluating the performance of a technique relies on dividing the noise term into several components, namely, reproducibility, repeatability and interobserver variation; and quantifying biological variation such as bodyweight, sex and age.

Reproducibility corresponds to between-day variability, whereas repeatability corresponds to within-day variability. The precise ultrasonographic evaluation of the length and width of the adrenal gland requires a good visualisation of the organ. A recent study reported that 91 per cent of the left and 86 per cent of the right adrenal glands could be visualised (n=100) (Barberet and others 2008). The right adrenal gland is more difficult to visualise than the left one because

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G. Mogenicato, PhD,

F. Raharison, PhD,

J. Sautet, PhD,

Anatomie – Imagerie Médicale –
Embryologie,

C. Layssol-Lamour, DVM,

F. Conchou, PhD,

A. Diquelou, PhD,

Groupe de Recherches pour Animaux
de Compagnie (GRAC),

D. Concordet, PhD,

UMR181 Physiopathologie et

Toxicologie Expérimentales, INRA,

École Nationale Vétérinaire de Toulouse,

Toulouse Cedex 3, France

E-mail for correspondence:

g.mogenicato@envt.fr

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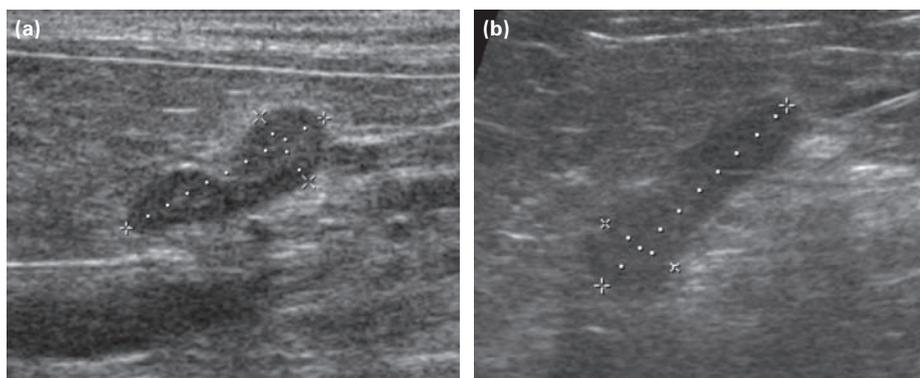


FIG 1: Longitudinal ultrasonography of the adrenal glands recorded in a conscious dog in dorsal recumbency for study 1. (a) Ultrasonography of the left adrenal gland illustrating its typical 'peanut' shape in the median plane. (b) Ultrasonography of the right adrenal gland. For both, adrenal length is represented by the '+' callipers and adrenal width is represented by the 'x' callipers

of its smaller size, deeper and more cranial location, and because of respiratory motion and the presence of gas in the pylorus, duodenum and colon.

Ultrasonography is highly observer-dependent (Saunders and others 1992), meaning that measurements can depend on the expertise of the observer. In published studies about adrenal ultrasonography, the conditions of the measurements of the length and width of the adrenal gland are always described technically, but relevant information, such as the number of observers involved and their level of expertise, is rarely provided. Such information would be extremely useful not only for interpreting the results of such studies but also for designing further studies using the same variables; for example, a study of the effect of a drug on the appearance of adrenal glands in dogs with PDH (Hörauf and Reusch 1999, Muller and others 2000, Ruckstuhl and others 2002). These variations of adrenal measurements must be known to quantify measurements in a large population of dogs.

Moreover, measurements vary according to biological variations such as age, sex and bodyweight. Douglass and others (1997) studied the relationship between adrenal size and the bodyweight, body surface area, age and sex of 193 dogs. The dogs were selected from the clinical population presented for routine abdominal ultrasonography and, therefore, a distinction between healthy dogs and dogs with non-adrenal diseases could not be made. Another study has differentiated healthy dogs, dogs with non-endocrine disease and dogs with untreated PDH, but with a small number ($n=20$) of animals for each category (Barthez and others 1995).

The aims of the present study were to evaluate the repeatability, reproducibility and observer-dependent variability of measurements of the left and right adrenal glands in healthy dogs; to quantify and analyse normal measurements of the adrenal glands in a large sample of conscious dogs by distinguishing healthy dogs from dogs with non-adrenal diseases, and to propose reference intervals for these two measurements; and to study the effects of bodyweight, age and sex on measurements of the adrenal glands.

Materials and methods

Dogs

The procedures used in this experiment were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). All dogs in both studies were determined to be healthy on the basis of a complete physical examination and plasma biochemistry (serum cholesterol and alkaline phosphatase levels), performed before inclusion in the study. All dogs with a clinical history of polyuria/polydipsia, clinical diagnosis of adrenal pathology or sonographic adrenal abnormality (eg, mass lesions or other abnormal architecture) were excluded. Age, breed, sex and bodyweight were recorded.

Ultrasonography of the adrenal glands

Longitudinal images of the adrenal glands were taken using a micro-convex (6 to 10 MHz) transducer. All ultrasound examinations were

carried out in fully conscious dogs, which were mainly in dorsal recumbency. Hair was clipped from the xiphoid process to the inguinal region. A large area was clipped to avoid any influence on the observer. Coupling gel was applied generously to this area of the abdomen. The technique for locating the adrenal glands was essentially the same as described by Nyland and others (2002). Adrenal measurements were performed from longitudinal views of the left and right adrenal glands. The gland was optimised in its long axis. The maximal length (from the cranial to the caudal pole of the gland) and width (either cranial or caudal pole) were determined by the sonographer using the unit's electronic callipers. If the adrenal gland was asymmetrical, the largest pole was measured (Fig 1).

Experimental design

Two separate studies were performed.

Study 1

The intra- and inter-day variabilities of the length and width of the left and right adrenal glands were determined by performing 270 (135 length and 135 width) right and 270 left examinations on five healthy beagle dogs (sexually intact males, mean [sd] age two [0.5] years, mean [sd] bodyweight 11.1 [0.2] kg) on three different days during a one-week period to avoid any biological time variation of the measurements. The dogs came from an accredited breeder of experimental animals (Avogadro).

Three observers with different levels of experience in ultrasonography performed the examinations. Observers 1, 2 and 3 were veterinarians with 10 years, four years and one year of training in ultrasonography, respectively. All observers were familiar with the ultrasonography examination procedure and the echograph.

Each day, the three observers examined all five dogs at three non-consecutive times, so each of them performed 30 examinations per day (15 examinations for the right adrenal gland and 15 examinations for the left). All of the examinations were randomised and blinded. For each examination, the observer took the measurements using callipers, and measurement values were hidden from the observer on the screen. Another person (not one of the three observers) was responsible for collecting all of the data.

Study 2

The study sample consisted of 146 dogs of different breeds seen at the clinic of the veterinary school of Toulouse between September 2004 and January 2006. The owner's consent for each dog was obtained before the its enrolment in the study. Dogs were classified into one of two categories, healthy or non-adrenal disease, defined as the 'status' of the dog. The clinically healthy dogs were those presented for vaccination. Dogs with non-adrenal disease included all dogs presented for any disease that was non-adrenal in nature; those with a clinical history of polyuria/polydipsia or a clinical diagnosis of adrenal pathology or sonographic abnormality were excluded. Ultrasonography of the right and left adrenal glands was performed by observer 1.

Statistical analysis

A software program (R version 2.10.0; R Foundation for Statistical Computing) was used to perform the analysis. The following linear mixed-effects model was used. For each observer and each ultrasonographic measurement (study 1):

$$Y_{ijk} = \mu + \text{Day}_i + \text{Dog}_j + \varepsilon_{ijk}$$

where Y_{ijk} is the k th value measured in dog j on day i , μ is the general mean, Dog_j is the fixed effect of dog j , Day_i is the random effect of the i th day and ε_{ijk} is the model error.

TABLE 1: Mean (sd) length and width of the left and right adrenal glands (mm) measured by each observer using ultrasonography in five conscious, healthy dogs

Observer	Left adrenal gland		Right adrenal gland	
	Length	Width	Length	Width
1	21.6 (0.5)	4.3 (0.1)	23.6 (1.0)	5.1 (0.1)
2	18.0 (0.5)	3.7 (0.1)	19.1 (0.4)	4.0 (0.1)
3	17.5 (0.3)	3.8 (0.1)	20.4 (0.9)	4.0 (0.2)

TABLE 2: Within-day and between-day sd and coefficient of variation (CV) values of measurements of the left and right adrenal glands by each of three observers, obtained by ultrasonography in five conscious, healthy dogs

Observer	Left adrenal gland		Right adrenal gland	
	Length	Width	Length	Width
1				
Within-day sd (mm)	1.6	0.5	1.8	0.6
Between-day sd (mm)	1.8	0.5	2.5	0.6
Within-day CV (%)	7.4 ^a	11.6 ^a	7.6 ^a	11.8 ^a
Between-day CV (%)	8.3 ^a	11.6 ^a	10.5 ^a	11.8 ^a
2				
Within-day sd (mm)	1.9	0.4	2.7	0.6
Between-day sd (mm)	2.0	0.4	2.7	0.6
Within-day CV (%)	10.6 ^a	10.8 ^a	14.1 ^b	15.0 ^b
Between-day CV (%)	11.2 ^a	10.8 ^a	14.1 ^b	15.8 ^b
3				
Within-day sd (mm)	2.2	0.5	2.8	1.1
Between-day sd (mm)	2.2	0.5	3.1	1.1
Within-day CV (%)	12.6 ^a	13.1 ^a	13.7 ^b	27.5 ^c
Between-day CV (%)	12.6 ^a	13.1 ^a	15.3 ^b	28.5 ^c

^{a, b, c} Superscript letters for CV that differ represent a significant difference ($P < 0.05$) between this measurement and the same measurement for another observer

The sd of repeatability and reproducibility was determined, respectively, as the residual sd and the sd of the day effect. Since the dogs included in the experiment were chosen a priori, the dog was considered a fixed-effects factor. The observer effect was quantified using the following general linear model.

$$Y_{ijkl} = \mu + \text{Day}_i + \text{Dog}_j + \text{Ob}_k + \varepsilon_{ijkl}$$

where Y_{ijkl} was the l th value measured for dog j on day i by observer k , μ is the general mean, Day_i and Dog_j have the same meaning as in the previous model, Ob_k is the differential effect (considered fixed) of observer k and ε_{ijkl} is the model error. The level of significance was set at $P < 0.05$.

Finally, a general linear model was used to analyse the effect of age, bodyweight, sex and status of the dog (ie, healthy or non-adrenal disease) on the ultrasonographic adrenal gland length and width (study 2). All of the effects were combined in a multiple regression analysis for the four variables: length of the left adrenal gland, width of the left adrenal gland, length of the right adrenal gland and width of the right adrenal gland. Reference intervals were then established according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2008).

Results

Study 1

Within-day and between-day intraobserver variability of the left adrenal measurements compared with the right

A mean of 94 per cent of the left (100 per cent for observer 1, 93 per cent for observer 2 and 89 per cent for observer 3) and 85 per cent of the right (98 per cent for observer 1, 84 per cent for observer 2 and 73 per cent for observer 3) adrenal glands were visualised.

Table 1 shows the length and width of the left and right adrenal glands, measured by each observer using ultrasonography, for each of the five beagles (in total, 270 measurements were attempted for each adrenal gland; the left was visualised on 254 measurements and the right on 230). There was no significant difference between the three observers for the lengths and widths of the left and right adrenal

glands. Table 2 shows the corresponding within-day and between-day variability results (sd and coefficient of variation). Overall, the results from all observers on all measurements showed mean sd values of 2 and 2.5 mm for the length of the left and right adrenal glands, respectively, and 0.5 and 0.7 mm for the width of the left and right adrenal glands, respectively.

The within-day and between-day coefficients of variation were not significantly different for the left adrenal gland measures; however, they were significantly different ($P < 0.001$) for the right adrenal gland between observer 1 and observers 2 and 3 for the length and between the three observers for the width.

The lowest within-day and between-day sd and coefficient of variation values were achieved by observer 1 for the length of the left and right adrenal glands. The largest within-day and between-day sd and coefficient of variation values were achieved by observer 3 for the width and length of the right adrenal gland.

Study 2

Demographic characteristics

Thirty-nine breeds were represented, the most common being the Yorkshire terrier ($n = 19$) and poodle ($n = 16$). Eight dogs were of mixed breed. The age ranged from one to 18 years (mean [sd] 7.9 [4.2]) and the bodyweight from 0.7 to 70 kg (mean [sd] 14.8 [12.1] kg). Fifty-nine were female (50 entire, nine neutered) and 87 were male (84 entire, three neutered); 40 were healthy and 106 had non-adrenal disease.

For this study, 96 per cent of the left and 86 per cent of the right adrenal glands were visualised (ie, the left adrenal gland was evaluated from ultrasonography in 141 dogs and the right adrenal gland in 126 dogs). The length of the left adrenal gland ranged from 10.3 to 33.7 mm (mean [sd] 19.8 [5.4] mm), whereas the width ranged from 2.3 to 7.7 mm (mean [sd] 5.3 [1.1] mm). The length of the right adrenal gland ranged from 9.4 to 30.7 mm (mean [sd] 18.3 [4.5] mm), whereas the width ranged from 3.0 to 10.5 mm (mean [sd] 5.7 [1.4] mm) (Table 3). The lengths and widths were not significantly different between the left and right adrenal glands. The measurements and reference intervals of the four variables are presented in Table 3.

Table 4 shows the effects of bodyweight, age, sex and status of the dogs for the left and right adrenal gland measurements. The lengths of the left and right adrenal glands were strongly correlated ($P < 0.0001$) with bodyweight, whereas for the width of the left adrenal gland there was a slight but statistically insignificant trend ($P = 0.0624$) and the width of the right adrenal gland was not correlated with bodyweight ($P = 0.953$).

The width of the left adrenal gland was strongly correlated with age ($P = 0.0019$). While the length of the left adrenal gland was also correlated with age, this difference was less significant ($P = 0.0388$). For the right adrenal gland, there was no correlation with age.

The width and length of the right adrenal gland were correlated with sex ($P < 0.05$). The width of the right adrenal gland is significantly larger in males and the length is significantly larger in females. There was no significant correlation between the length or width of the left adrenal gland and sex. There was no significant effect of the status of the dog for the length and width of the left or right adrenal glands.

Discussion

This study provides data on the adrenal measurements of conscious dogs obtained using ultrasonography. The methods for locating and echographing the adrenal glands have been used in previous studies (Kantrowitz and others 1986, Barthez and others 1995, Douglass and others 1997).

The right adrenal gland was more difficult to image, as several previous studies have found (Barthez and others 1995, Grooters and others 1996, Douglass and others 1997, Tidwell and others 1997, Nyland and others 2002, Mantis and others 2003, Barberet and others 2008). Indeed, in the present study, the cranial pole was sometimes not very visible and, therefore, it was difficult to measure (Fig 1). This relative difficulty is because of gas in the gastric pylorus, duodenum and ascending colon (Tidwell and others 1997, Nyland and others 2002), although the percentage of visualisation of the right adrenal

TABLE 3: Mean (sd), ranges and reference intervals of measurements of the left and right adrenal glands obtained by ultrasonography in dogs from study 2

	Left adrenal gland (n=141)		Right adrenal gland (n=126)	
	Length (mm)	Width (mm)	Length (mm)	Width (mm)
Mean (sd)	19.8 (5.4)	5.3 (1.1)	18.3 (4.5)	5.7 (1.4)
Min-max	10.3-33.7	2.3-7.7	9.4-30.7	3.0-10.5
Reference interval	11.8-32.5	3.4-7.5	11.1-29.5	3.3-9.2

TABLE 4: P values for the variability of measurements of the length and width of the left and right adrenal glands in dogs of different bodyweight, age, sex and status, as measured by ultrasonography in study 2

Effects	Left adrenal gland (n=141)		Right adrenal gland (n=126)	
	Length	Width	Length	Width
Bodyweight	<0.0001	0.0624	<0.0001	0.953
Age	0.0388	0.0019	0.536	0.808
Sex	0.342	0.613	0.0475	0.0392
Status	0.901	0.718	0.511	0.461

increased with the level of training of the observer. This difficulty partly explains the difference in sd values between the three observers' measurements (lowest for the most trained observer and highest for the least trained) and the coefficient of variation between the left and right adrenal glands: again, lower for a trained observer.

Before an organ is measured, for example, to determine the effect a drug will have, it is important to know the repeatability and reproducibility of the measurement of the organ. The coefficient of variation is usually used for within-day and between-day variabilities. However, it is interesting to know the difference between two or three repeated measurements of the same animal by using sd. Indeed, if this difference is important, the obtained results of an organ's measurements must be interpreted with care. Moreover, the within-day and between-day variabilities are different from one observer to another.

The sd values presented in Table 2 were rather low in comparison to the values for mean lengths and widths, suggesting little intraobserver variation; however, interobserver data show that the measured length and the width of both adrenal glands generally increased with the training of the observer (Table 1). This is likely to be because a more experienced observer is able to visualise more of the adrenal gland on the ultrasound image. The within-day sd values for the widths of the left and right adrenal glands were about equal for observer 1, increased by 50 per cent between the left and right widths for observer 2 and multiplied by two for observer 3. Although less pronounced, there were also increases in the within-day sd values between left and right measurements for the length. Taken along with the data showing that the left adrenal gland was visualised more easily, these results suggest that the width or length of the left adrenal gland are good indicators to study the modification of adrenal gland size.

The results of the two studies showed great similarity between the mean measurements and reference intervals for the left and right adrenal glands. Study 2 also showed very little or no correlation between bodyweight or sex and the widths of the left and right adrenal glands, whereas there was a strong correlation between bodyweight and the lengths of the left and right adrenal glands. The relatively weak correlations between sex and both length and width of the right adrenal gland have no physiological basis and these correlations are likely to be a random effect. Due to the significant effect of bodyweight on the length of the left adrenal gland, but not its width, the authors conclude that the width of the left adrenal gland should be chosen for evaluation and measurement of adrenal glands in healthy dogs. The results reinforce a previous study that recommended using this measurement to assess adrenal gland size in dogs with a significant likelihood of having PDH (Barthez and others 1995).

Barthez and others (1995) also suggested a decision limit of 7.5 mm for the width of the left adrenal gland, meaning that a dog with a left adrenal gland width of greater than 7.5 mm was likely to have PDH.

This matches the upper limit of the reference interval determined in the present study for the width of the left adrenal gland in healthy dogs (Table 3). It should therefore be noted that the inclusion of dogs with PDH in future studies would influence the reference interval. Study 2 showed a correlation between age and the width of the left adrenal gland (Table 4). Systemic diseases are known to potentially induce adrenal enlargement, which could explain the effect of age on adrenal size (Grooters and others 1996, Douglass and others 1997) since older dogs are more likely to have a systemic disease. Thus, the greatest care must be taken to avoid including false-negative or false-positive results when attributing a diagnosis of PDH because the increase in size could be due to another condition. On this subject, Barthez and others (1995) showed, using transverse adrenal measurements, an 81 per cent sensitivity and 100 per cent specificity for differentiating normal dogs from dogs with PDH, and so this limit of 7.5 mm seems to be a good compromise while keeping in mind the potential effect of age. However, if the dog is showing clinical and biological signs of having PDH, both adrenal glands must be visualised before drawing any conclusions.

Study 2 showed that there was no significant difference in the length or width of the left or right adrenal glands between the population of healthy dogs and the population of dogs with non-adrenal disease. It would be interesting to compare two large populations of dogs – one population of 'healthy' dogs (with or without non-adrenal disease) and another of dogs with PDH – to confirm the diagnostic effectiveness of the proposed upper limit of 7.5 mm and to re-evaluate the influence of age on the reference interval. Indeed, the study that proposed this limit value compared only 40 'healthy' dogs with 20 dogs with PDH (Barthez and others 1995).

The results demonstrate that, as with any quantitative technique, adrenal gland measurements should be validated for a given observer, and a well-trained and experienced observer will deliver the best performance. When measuring adrenal glands, the sd and the coefficient of variation values are smallest for the left adrenal gland width. This measurement is therefore of most interest when assessing the adrenal glands of dogs. Moreover, this variable is not significantly correlated with bodyweight or sex. However, the effect of age on this value must be taken into consideration. The present study also showed no significant differences between healthy dogs and dogs with non-adrenal disease. This information should be taken into account for further studies comparing two populations of dogs to discover the upper or lower limits of adrenal size for healthy dogs because it shows that dogs with non-adrenal disease can be included in the 'healthy' group.

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