Article

Computed Tomographic Assessment of Pituitary Gland Dimensions in Domestic Short-Haired Cats.

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Simple Summary: The pituitary gland is crucial in regulating metabolic processes. Tumors in this gland can be subtle, making establishing reference values for pituitary dimension pivotal. Computed tomography is commonly used for identifying pituitary alterations, for planning surgery or radiation therapy and monitoring treatment response. This study aimed to determine the normal pituitary size and the pituitary-to-brain ratio in a group of domestic short-haired cats by computed tomography. The study also aimed to explore the correlations between body weight, age, sex, and pituitary dimensions and to assess the inter- and intra-agreement between different operators in measuring pituitary dimensions. The study showed that the normal range for pituitary dimensions is wider than previously reported. The study also showed a low correlation between body weight, age, and pituitary dimensions. The intra-operator agreement in measuring pituitary dimensions was good/excellent, but the inter-operator agreement was moderate/good, likely due to differences in expertise. The reference values obtained from this study can help evaluate the pituitary gland size in domestic short-haired cats with suspected pituitary neoplastic lesions, aid in surgical or radiation therapy planning and monitor treatment response.

Abstract: The detection of subtle changes in the pituitary dimensions has relevant implications since this gland is crucial for the endocrine system. In cats, few studies established the cut-offs values of the pituitary gland's dimensions, but using small and inhomogeneous samples. The aims of this study were: to determine by computed tomography (CT) the pituitary linear dimensions and the pituitary-to-brain (P:B) ratio in a sample of domestic short-haired (DSH) cats; to assess the effects of sex, age and weight on pituitary dimensions; to evaluate the inter- and intra-observer agreement for such measurements. Exclusion criteria were: clinical, laboratory or CT alterations of pituitary gland or brain diseases, fractures of the neurocranium, or diabetes mellitus. Pituitary dimensions and brain area were assessed by two different observers using multiplanar reconstructions and automated segmentation tools. Fifty-one cats were included in the final sample. The intraclass correlation coefficient for intra- and inter-observer reliability showed good/excellent and moderate/good reliability, respectively. No differences between sexes were detected, and negligible correlations were found with age and weight. Findings from the current study provided normal reference values for pituitary height (1.88 – 4.01 mm) and P:B ratio (0.25 – 0.49), useful for assessing abnormally enlarged pituitary gland in DSH cats.

Keywords: acromegaly; adenoma; diabetes; feline; hypophysis; microadenoma.

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1. Introduction

The pituitary gland plays a pivotal role in regulating the endocrine system through the production, storage, and release of various hormones [1]. Pituitary tumors can originate from different cell lineages and can be either functional or non-functional [2-6]. The clinical signs associated with the neoplasm depend on the secretory proprieties but also on the tumor's size [2,4,6-9]. Indeed, also non-functional tumors can become clinically relevant when they enlarge enough to cause neurologic signs by direct compression of other intracranial structures [3-6,10]. Furthermore, pituitary tumors can be incidental findings when skull imaging is performed for unrelated reasons (so-called incidentalomas) [8]. In humans, according to 2017 WHO "classification of tumors of the pituitary gland", pituitary neoplasms can be classified as adenomas or carcinomas. The latter are defined only by demonstrating metastatic spread [5]. This classification mainly focuses on immunohistochemical classification according to the hormone that the tumor expresses and so can be further subdivided into melanotroph, corticotroph, thyrotroph, lactotroph, somatotroph or gonadotroph. In dogs, corticotroph (ACTH-secreting) adenomas and adenocarcinomas responsible for pituitary-dependent hyperadrenocorticism prevail, while in cats, somatotroph (growth hormone – GH – secreting) tumors are the most reported [6]. In the latter case, the excessive secretion of GH can result in chronic hypersomatotropism that can cause acromegaly and insulin resistance, due to the concomitant increase of insulin-like growth factor-1 (IGF-1). Of note, cats affected by somatotroph pituitary tumors are often brought to consultation for clinical signs related to poorly controlled diabetes, such as polyuria, polydipsia and polyphagia, rather than somatic changes due to acromegaly [10-12]. Somatotroph adenoma is the most reported pituitary tumor in middle-aged to older male cats. Domestic short-haired (DSH) cats and Maine Coons seem predisposed [10-12].

The detection of anatomical alterations of the pituitary gland is usually performed using Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) [7]. These imaging techniques are paramount for diagnosis and for surgical planning or radiotherapy [8]. In many cases, the diagnosis of pituitary macroadenoma is straightforward since the pituitary mass dorsally protrudes from the sella turcica and compresses the adjacent brain parenchyma, sometimes with associated neurological signs [7-9]. Differently, in the case of microadenomas, there are often only subtle changes in pituitary size and contours that may not be visible [9,12]. In order to increase diagnostic accuracy in the detection of pituitary microadenomas, the pituitary gland was also evaluated using CT dynamic scans, since pituitary tumors can alter the normal network of pituitary vessels and, consequently, the enhancement pattern [13].

Few studies tried to establish the linear dimensions of the pituitary gland (height, width, and length) in cats using either MRI [14] or CT [13,15,16]. In order to address variation in pituitary gland size between dogs and cats of different sizes and breeds, the pituitary height-to-brain ratio (P:B ratio) was introduced [15-17]. However, those studies performed in cats used third-generation CT devices [15] or thick MRI slices [14] and included small and inhomogeneous samples. More recently, a study aimed to evaluate possible differences in pituitary size between mesaticephalic and brachycephalic cats found a significant difference between the two skull morphotypes [16]. These results highlight the need to obtain reference measures of the pituitary gland according to morphotype or, even better, according to the breed. To date, a single study, including only DSH cats, described the pituitary dimensions and P:B ratio [15]. However, in our clinical experience, cats without hematological, clinical and CT findings of pituitary disease had pituitary dimensions outside the previously proposed cut-off values. Consequently, we hypothesized that the reference intervals for pituitary linear dimensions (height, length, and width) and the P:B ratio in DSH cats would be different than previously reported.

Therefore, the primary aim of the present study was to verify if the previously reported cut-off values for pituitary linear dimension and P:B ratio were respected in a larger sample of DSH cats and eventually, establish new reference values. Secondary

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objectives were to evaluate the influence of age, body weight and sex on pituitary dimensions and P:B ratio. Finally, we evaluated the intra- and inter-observer agreement among two observers with different levels of expertise.

2. Materials and Methods

2.1. Selection and description of subjects

This single-center, retrospective, reference interval, intra- and inter-observer agreement study was approved by the Ethical Animal Care and Use Committee of the University of Napoli "Federico II" (Prot. N. PG/2023/0050567). The electronic clinical records and CT studies of cats referred to the Interdepartmental Centre of Veterinary Radiology of the University of Napoli Federico II in the set study period between September 2018 and October 2022 were retrieved from the picture archiving and communication system (dcm4chee-arc-light version 5.11.1, http://www.dcm4che.org) [18]. The inclusion criteria were: DSH breed, same CT unit and scanning protocol. Patients were excluded from the study if they had clinical, laboratory, or CT final report referring any alteration related to a) pituitary gland disease, b) neurologic signs different from vestibular symptoms, c) intracranial lesion detected on CT, d) fractures or conformational alterations of the neurocranium, e) polyuria or polydipsia, f) signs of acromegaly, d) definitive diagnosis of diabetes mellitus.

2.2. CT scan protocol

All patients were positioned in sternal recumbency within a radiolucent polyurethane vacuum immobilization mattress (Vacuumat, Génia, St. Hilaire de Chaléons, France) and with the forelimbs pulled caudally along the thorax, under general anesthesia (the anesthetic protocol adopted varied depending on the decision of the anesthesiologist in charge). Computed tomography studies were obtained using a 16-slices multi-detector computed tomography (MDCT) unit (BrightSpeed, General Electric Healthcare, Milwaukee, WI, USA). The acquisition protocol was: helical mode; slice thickness 1.25 mm; pitch 0.9375:1; 120 kVp, 160-200 mA, 1-second tube rotation speed; soft tissue and bone reconstruction algorithms (General Electric proprietary "standard" and "bone" filters). All the patients received a standardized intravenous dose (740 mgI/kg, i.e., 2 ml/kg) of contrast media (Iopamidol, Iopamiro 370 mgI/ml, Bracco Imaging s.p.a., Milano, Italy) using an infusion rate of 1 ml/s followed by a 5 ml saline flush through a double-barrel power injector (EmpowerCTA+, Bracco Imaging s.p.a., Milano, Italy); post-contrast images were acquired after a fixed delay of 60 seconds.

2.3. Data recording and image analysis

A preliminary evaluation was performed by a Veterinary radiologist (L.M., full professor of Veterinary Radiology with a Ph.D. and >25 years of experience) to include or exclude each cat from the definitive sample group. The CT studies were excluded if: a) the postcontrast series of the skull acquired with soft tissue reconstruction algorithm was not available, b) the images quality was inadequate for a correct interpretation, c) the presence of beam hardening artifacts was detected, preventing a correct evaluation of the pituitary gland. The same author anonymized all the CT studies before submitting them to two observers who reviewed the images using a commercial DICOM viewer software (Philips Extended Brilliance Workspace v. 4.5.5, Philips Medical System Nederland B.V., Best, The Netherlands). Observer 1 (D.C., a third-year Ph.D. student in Veterinary Diagnostic Imaging) and observer 2 (P.C, a veterinarian with two years of expertise in CT imaging) were blinded regarding the clinical data and reasons for CT examination. All the measurements were performed once by each observer, independently and blinded to the results reported by the other observer. A pituitary gland measurement method was established prior to the analysis by two authors (D.C. and L.M) and recorded on a portable document file. Pituitary linear dimensions (height, length, width) expressed in millimeters (mm) were

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measured using electronic calipers on post-contrast images displayed using a standardized window [window width (WW): 450, window level (WL): 200], although WW and WL could be manually adjusted by the observers if deemed necessary. Measurements were made on multiplanar reconstructions (MPR) images in order to obtain the best visualization of the pituitary gland and avoid interpreting the dorsum sellae as a pituitary mass [9]. The pituitary height (PH) was measured at the level of the pituitary fossa, perpendicular to the basisphenoid bone, where the maximal pituitary height was visible, both on the transverse plane (PHT) (Figure 1A) and on the sagittal plane (PHS) (Figure 1B). The pituitary length (PL) was measured on the sagittal plane where the maximal length was visible, parallel to the basisphenoid bone (Figure 1B). The pituitary width (PW) was determined on the transverse plane at the point of maximal width of the gland (Figure 1C). The brain area (BA) expressed in mm^2 was measured on the same slice of the PHT using an automated segmentation tool (Figure 1D). When deemed necessary, the observer used the bone reconstruction algorithms with associated high contrast window (WW = 2000, WL = 800) to better delineate the brain edges. All the data were reported in an electronic spreadsheet (Microsoft Excel version 16.52 2021, Microsoft Corp. Redmond, WA, USA) and the P:B ratio was automatically computed for each cat as follow: P:B ratio = [PHT(mm)×100/BA(mm^2)] [17]. To assess the intra-observer agreement, the measurements were repeated independently by the two observers two months after the first evaluation on a smaller sample of thirty re-anonymized and re-randomly selected CT exams. The sex, neutering status, weight (in kilograms), and age (in months) were recorded for each cat included in the final sample group.

2.4. Statistical analysis

Statistical analyses were performed by one of the authors (L.A., former researcher with a Ph.D. and >10 years of experience and training in statistics) using commercial software (JMP Pro, v. 16.0, SAS Institute, Cary, NC, USA; MedCalc version 19.2.6, MedCalc Software Ltd, Ostend, Belgium; IBM SPSS, v. 26.0, IBM, Armonk, NY, USA). The normality of data was assessed with the Shapiro-Wilk's W test. Continuous data were reported as mean ± standard deviation (SD) or median (range) depending on the distribution.

The reference range for each measurement was calculated according to the American Society for Veterinary Clinical Pathology (ASCVP) guidelines for reference intervals [19]. Briefly, outliers were automatically identified according to Reed *et al*. [20]. Then, data distribution was tested automatically with the Shapiro-Wilk's W test. Reference lower and upper limits, and the corresponding 90% confidence intervals (CIs) were then calculated employing the robust method following Clinical Laboratory and Standards Institute (CLSI) recommendations (CLSI C28-A3), with bootstrapping (1000 iterations) [21]. A Bland-Altman plot was used to explore the differences between PHS and PHT. The bias and 95% limits of agreement were calculated. Correlations between the measurements and body weight were tested with Pearson's correlation coefficient (r), whereas those between the measurements and age using Spearman's rank correlation coefficient (r_s). Finally, differences between males and females were tested with a pooled Student's t-test, with Welch's correction since variances resulted significantly different at the F test (PL, PW, BA, and P:B ratio), or a Mann-Whitney's U test (PHT and PHS), according to within sex distribution and non-considering the neutering status. No partitioning into subclasses based on age, body weight or sex was applied in the reference range calculations due to the lack of correlation with the measurements.

For inter-observer reliability, measurements belonging to all the patients were included in the analysis, while for the intra-observer reliability, only the measurements of the smaller sample were considered. For both inter- and intra-observer agreement, a two-way mixed effects intraclass correlation coefficient (ICC) for single measurement or single observer accordingly, and absolute agreement was calculated; the relative 95% CI were calculated, as well. The ICC was categorized according to Koo and Li. [22]. Based on the reliability analysis results, all intra- and inter-observer measurements with an ICC > 0.80

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were averaged and subsequently analyzed. For measurements that did not reach this value, the first measurements of observer 1, considered the most experienced, were used. In all analyses, $p < .05$ was considered statistically significant.

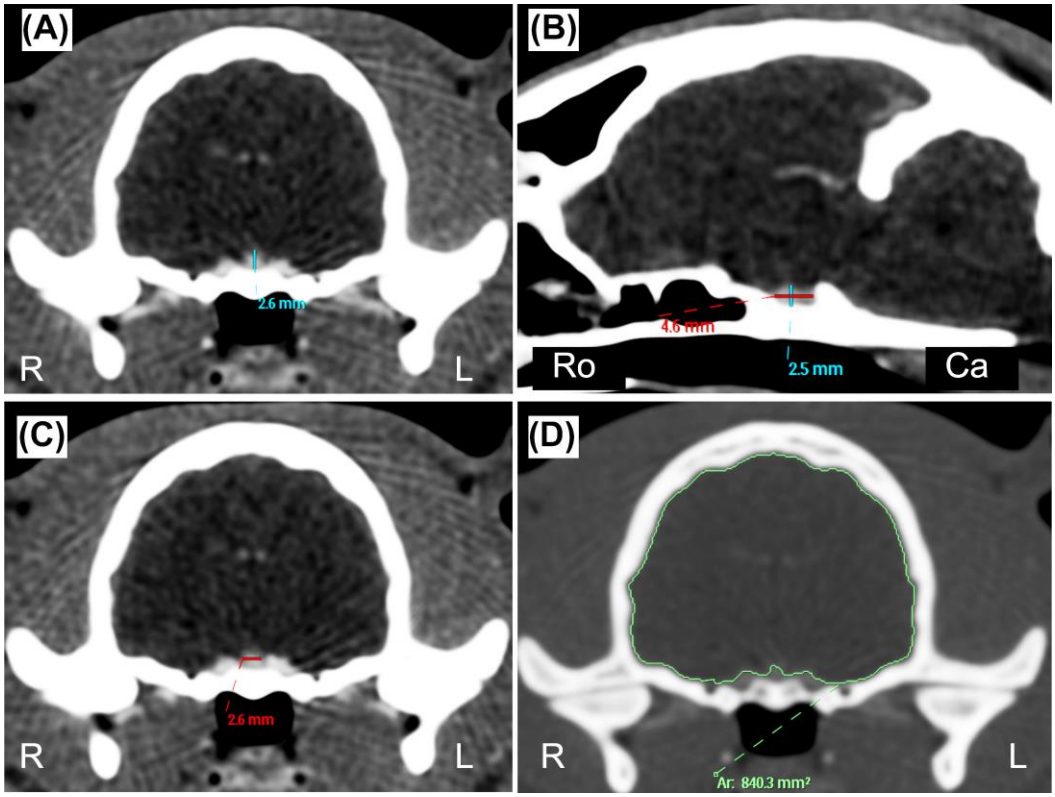

Figure 1. (A, C, D) Transverse and **(B)** sagittal soft tissue algorithm postcontrast CT images of the skull of a twelve-month-old DSH cat. **(A)** Pituitary height (blue line) measured on the transverse plane (PHT) at the level of the pituitary fossa, perpendicular to the basisphenoid bone. **(B)** Pituitary height (blue line) measured on the sagittal plane (PHS) perpendicular to the basisphenoid bone and pituitary length (PL, red line) measured where the maximal length of the pituitary gland was visible and parallel to the basisphenoid bone. **(C)** Pituitary width (red line) measured on the transverse plane (PW) at the point of maximal width of the gland. **(D)** Brain area (BA, green line) measured on the transverse plane, at the same level of the PTH using an automated region of interest tool. **(A, B, C)** manually windowed to WW = 455, WL = 234; **(D)** manually windowed to WW = 2000, WL = 800. Abbreviations: Ca: Caudal; L: Left; R: Right; Ro: Rostral.

3. Results

A total of 59 CT studies performed in the set period met the inclusion criteria. After the preliminary review, eight cases were excluded for the following reasons: brain neoplasia ($n = 4$), deformed cranium ($n = 2$), and lack of post-contrast images ($n = 2$). Fifty-one DSH cats were included in the final sample; they were 4 intact females (8%), 26 (51%) spayed females, 7 (14%) intact males, and 14 (27%) castrated males. The median age was 72 months (range 2 – 180), and the weight 4.6 ± 1.5 kg.

In all the cats included in the final sample, the pituitary gland was distinguishable from the adjacent structures with a good contrast enhancement and all the predetermined measurements were obtained. In three cases it was challenging to identify the pituitary edges with absolute precision due to the pituitary gland's small size. The automated segmentation tool allowed for a rapid delimitation of the BA in all cases.

All the measurements described resulted normally distributed, and no outliers were

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detected in any of them. The reference intervals, mean ± SD and range (minimum to maximum) for linear measurements and P:B ratio are reported in Table 1.

Table 1. Mean ± standard deviation, upper and lower limits of the reference value (and corresponding 90% CI) of the pituitary gland linear measurements, brain area and pituitary-to-brain ratio.

Measurement	Mean (±SD)	Range (Min–Max)	Lower Limit (90% CI)	Upper Limit (90% CI
PHT (mm)	2.94 (± 0.52)	2.0 – 4.1	1.88 (1.68 – 2.10)	4.01 (3.81 – 4.20)
PHS (mm)	2.95 (± 0.55)	1.9 – 4.0	1.84 (1.63 – 2.08)	4.10 (3.89 – 4.27)
PL (mm)	3.17 (± 0.52)	2.0 – 4.4	2.13 (1.92 – 2.37)	4.23 (4.03 – 4.42)
PW (mm)	3.24 (± 0.61)	2.1 – 4.8	1.92 (1.66 – 2.15)	4.41 (4.12 – 4.71)
BA (mm^2)	789.02 (± 61.85)	647.87 – 962.85	659.51 (634.51 – 687.61)	910.96 (883 – 938.01)
P:B ratio	0.37 (± 0.06)	0.25 – 0.48	0.25 (0.23 – 0.28)	0.49 (0.47 – 0.51)

Abbreviations: CI, confidence interval; BA, brain area; Max, maximum; Min, minimum; P:B ratio, pituitary gland height to brain area ratio; PHS, maximal pituitary height on the sagittal plane; PHT, maximal pituitary height on the transverse plane; PL: pituitary length; PW: pituitary width; SD, standard deviation

Regarding the pituitary height, the mean (±SD) PHT was 2.94 ± 0.52 mm and the PHS 2.95 ± 0.55 mm, and the Bland-Altman evaluation resulted in a bias of -0.01 and limits of agreement of -0.79 – 0.76 (Figure 2).

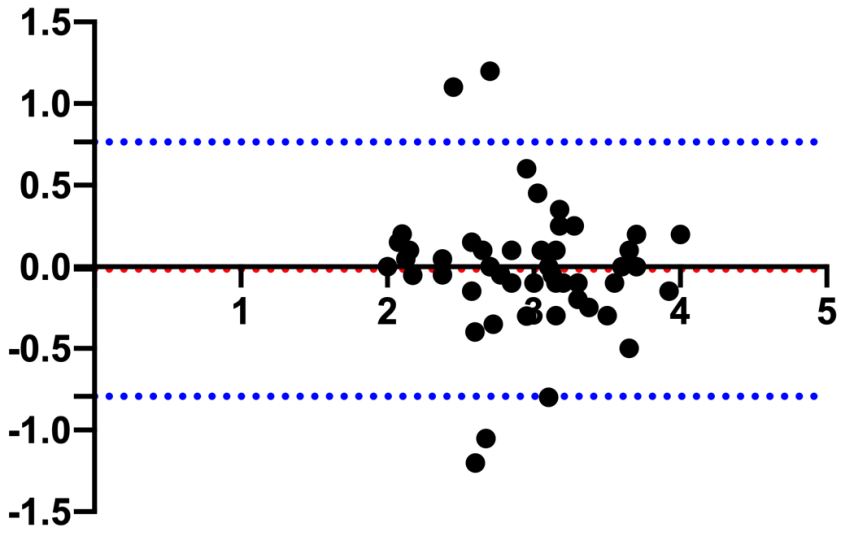

Figure 2. Bland-Altman plot comparing measurements of the pituitary gland height measured in the transverse (PHT) and sagittal (PHS) plane. The y-axis shows the difference between the two measurements, and the x-axis shows the average. The blue dotted lines represent the 95% confidence intervals, and the red dotted line represents the bias.

All measurements showed negligible to low correlation with body weight, whereas only PHS and PL showed a low correlation with age. Correlation coefficients and relative *p*-values are summarized in Table 2. No differences between sexes were detected for any

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measurement (PHT, $p = 0.40$; PHS, $p = 0.68$; PL, $p = 0.31$; PW, $p = 0.21$; BA, $p = 0.30$; P:B ratio, $p = 0.94$).

Table 2. Mean ± standard deviation, upper and lower limits of the reference value (and corresponding 90% CI) of the pituitary gland linear measurements, brain area and pituitary-to-brain ratio.

Measurement	Body weight	*p*-value	Age	*p*-value
PHT (mm)	r = 0.39	0.004	r_s = 0.22	0.12
PHS (mm)	r = 0.30	0.032	r_s = 0.35	0.012
PL (mm)	r = 0.28	0.044	r_s = 0.36	0.009
PW (mm)	r = 0.35	0.011	r_s = 0.12	0.39
BA (mm^2)	r = 0.29	0.036	r_s = 0.17	0.24
P:B ratio	r = 0.29	0.038	r_s = 0.14	0.33

Abbreviations: CI, confidence interval; BA, brain area; P:B ratio, pituitary gland height to brain area ratio; PHS, maximal pituitary height on the sagittal plane; PHT, maximal pituitary height on the transverse plane; PL: pituitary length; PW: pituitary width; r: Pearson's correlation coefficient; r_s: Spearman's rank correlation coefficient.

Results for inter-observer agreement are summarized in Table 3. All measurements were within the moderate reliability class, but the BA for which good reliability was detected.

Table 3. Intraclass correlation coefficients (ICC), relative 95% confidence intervals and *p*-values for the inter-observer reliability test.

Measurement	ICC	95% CI	*p*-value
PHT	0.69	0.38 – 0.84	< 0.0001
PHS	0.58	0.37 – 0.74	< 0.0001
PL	0.58	0.36 – 0.73	< 0.0001
PW	0.66	0.46 – 0.79	< 0.0001
BA	0.81	0.50 – 0.91	< 0.0001

Abbreviations: CI, confidence interval; BA, brain area; ICC, intraclass correlation coefficients; PHT: pituitary height in the transverse plane; PHS: pituitary height in the sagittal plane; PL: pituitary length; PW: pituitary width.

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Results for the intra-observer agreement are summarized in Table 4. Reliability was good for all measurements from observer 1, but for PL that resulted moderate; on the other hand, reliability resulted moderate for all measurements from observer 2, but for BA that resulted excellent.

Table 4. Intraclass correlation coefficients (ICC), relative 95% confidence intervals and *p*-values for the intra-observer reliability test.

Measurement	Operator 1			Operator 2		
	ICC	95% CI	p-value	ICC	95% CI	p-value
PHT	0.81	0.63 – 0.91	< 0.0001	0.66	0.17 – 0.86	<0.0001
PHS	0.87	0.71 – 0.94	< 0.0001	0.60	-0.04 – 0.85	<0.0001
PL	0.51	0.19 – 0.73	0.001	0.70	0.10 – 0.89	<0.0001
PW	0.78	0.59 – 0.89	< 0.0001	0.60	0.08 – 0.83	<0.0001
BA	0.82	0.66 – 0.91	< 0.0001	0.92	0.84 – 0.96	<0.0001

Abbreviations: CI, confidence interval; BA, brain area; ICC, intraclass correlation coefficient; PHS, maximal pituitary height on the sagittal plane; PHT, maximal pituitary height on the transverse plane; PL: pituitary length; PW: pituitary width.

4. Discussion

The primary aim of the present study was to verify if the previously reported cut-off values for pituitary linear dimension and P:B ratio were respected in a larger sample of DSH cats and eventually, establish new reference values. The obtained values partly differ from those already reported in the literature [13-16]. This discrepancy might be related to differences in sample sizes. Effectively, to obtain reliable reference values, a sample size of 120 healthy patients should be collected [19,20,23]; nonetheless, robust statistical methods have been developed for sample sizes less than 120 and more than 40, such as that used in our work, which allow the calculation of the reference limits and 90% CI [19]. In this study the reference values for the pituitary linear dimensions and P:B ratio were found according to the guidelines of the ASCVP [19]. Calculation of the reference limits and 90% CI for sample sizes less than 40 have been discouraged [19,23].

Another possible cause of discrepancy between reported values and those from the current study, may be the use of different imaging techniques (CT vs. MRI), different CT devices or scan protocols. It is well known that MRI has a higher soft tissues contrast compared to CT, that might give more accurate and reproducible results [7,24]. On the other hand, older MRI devices might not have enough field strength, or precise electronic calipers in the range of millemeters [11]. Differences between CT devices, scan protocols [25,26], or even contrast dosage and infusion rate [27], can all affect images quality and, consequently, correctness, reproducibility and accuracy of the measurements. In the current study, all the CT studies were performed using a MDCT unit providing high spatial resolution and real isotropic reconstructions [26]. These technical aspects gave true MPRs and allowed precise assessment of the pituitary gland [28].

Moreover, we selected only DSH cats, reducing an eventual inter-breeds variability. A significant difference in pituitary gland linear measures between brachycephalic and mesaticephalic cats has already been demonstrated [16], therefore, it is reasonable hypothesize that other factors, e.g. the breed, might have some influence on those measures. Larger samples of cats stratified by breed might confirm this hypothesis.

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In other studies [11,14], both PHT and PHS have been reported, whereas Nadimi *et al.*, reported only the PHT [15]. In the current study, the Bland-Altman plot showed minimal bias (- 0.01) between PHT and PHS. This finding represents an indirect validation of the accuracy of the measurements reported, further confirmed by the differences in all the reported statistics (mean, SD, range, reference interval and 90% CI) that deviate from each other in the order of 0.1 mm. Therefore, according to us, both the transversal and the sagittal scans can be used interchangeably to determine the pituitary height.

Partitioning, i.e. the stratification into sub-groups, is an important step during reference range evaluation [19], as well as in other cohort studies [29]. Sub-groups might be planned in advance based on expected physiological differences, e.g. due to sex and age, or might be identified during data analysis. In the latter case, a strong statistical result should justify the stratification [29].

One of the secondary objectives of this work was to evaluate the influence of age, body weight and sex on the pituitary measurements, also to evaluate if any sub-group had to be generated. All the linear measurements had negligible/low correlation with body weight, and only PHS and PL had a low correlation with age. In a previous study, performed on fifteen cats [15], ten of which were DSH, the authors found a significant correlation between pituitary dimensions and body weight; however, the degree of this correlation was not reported. Additionally, considering only the DSH cats, the authors reported a significant difference between those weighing < 3 kg and those above that body weight [15]. In our study, although body weight was positively correlated with all pituitary dimensions, the degree of correlation was negligible/low, in partial agreement with other studies [14,16]. Therefore, we did not consider it worth of creating subgroups based on body weight. Further studies, with larger sample sizes should explore this association. Nonetheless, in the authors' opinion, the body weight per se might not be the best variable to be explored. Perharps, other body metrics, e.g. the body surface, body condition score, etc., could act as a better predictor of differences in pituitary gland dimensions. However, the P:B ratio might eliminate any influence of body weight and dimensions.

In humans it is well-known the progressive growth of the pituitary gland during puberty [30,31] and the small size of the pituitary gland in elderly people [32]. In cats, Häußler *et al*. [16] found a positive correlation between age and pituitary height, width and length in the sub-group of brachycephalic cats, but 25% of them was less than 15 months, whereas this correlation was not explored in the mesaticephalic group. In our sample, 11 cats (21%) were less than 15 months old, but we detected only a low correlation of PHS and PL with age. Consequently, no conclusions can be drawn about the different results reported. Further studies should be designed to explore whether in the feline species a pattern of pituitary growth and late reduction in size, similar to that of human beings, exists.

Finally, no correlation was found between gender and pituitary dimensions. This finding agrees with a previous study [15], but partially disagrees with the study from Häußler *et al*. [16] where male cats had significantly larger pituitary width compared to female. However, the authors did not show the correlation coefficient, and significance was reached only in the mesaticephalic sub-group. In our study, the high number of neutered females and castrated males, which compose more than two third of the whole sample (78%), might have introduced a bias, even if the neutering status has been reported to have no influence on the pituitary dimensions [13]. Again, further studies with homogeneous gender larger groups are needed to clarify the influence of gender and neutering on the pituitary gland dimensions.

The other secondary objective was the evaluation of the intra- and inter-observer agreement. Results of the ICC for the intra-observer agreement suggest that it was influenced mainly by the experience of the operator. Indeed, the observer 1, considered the most experienced, showed good reliability and, except for PL and BA, displayed higher ICC values compared to observer 2. The ICC for inter-observer agreement revealed a moderate/good agreement between observers. In a previous study, assessing the intra- and

 doi:10.20944/preprints202305.0505.v1

interobserver agreement accuracy and reproducibility of CT measurements of the pituitary gland in dogs on a phantom model, the authors found an excellent level of agreement for PH and P:B ratio between the observers [33]. However, a systematic and significant difference was present between them. The authors concluded that due to this systematic variation, intra- and inter-patient comparisons have to be performed preferably by the same observer [33]. In another study, Van Hoe *et al*. suggested a fundamental role of image windowing in the manual measurement of small parts [34]. Accordingly, in the current study, notwithstanding the suggested pre-defined windowing, the moderate agreement between observers may rely on the operator's experience and ability in setting the window level and width, when it was considered necessary to better measure the pituitary gland. The ICC results for BA indirectly supports this hypothesis. Indeed, the BA was the measurement with the higher level of agreement between observers and this is probably related to the automated segmentation tool used, thus eliminating the operator dependence.

The main limitation of this study is the absence of IGF-1 assay in cats included in the final sample. Consequently, it is impossible to exclude categorically acromegaly. However, none of the subjects had clinical, CT or laboratory findings consistent with acromegaly or diabetes mellitus. Given the small number of laboratories that perform this analysis and the relatively high cost, the assay for IGF-1 is not performed as part of the routine serum analysis panel at our institution, as it is reserved for cats with clinical suspicion of acromegaly. Another limitation is the absence of necropsy and, therefore, the possibility of ruling out pituitary lesions and also to verify the agreement between the actual ex vivo dimensions of the pituitary gland and those obtained on CT images.

5. Conclusions

This study provides reference values for pituitary dimensions and P:B ratio in DSH cats. The reference values are wider than the mean values previously reported. Pituitary linear dimensions and P:B ratio exhibit a good intra-operator agreement, but a moderate inter-operator agreement, likely due to the different expertise. Software that automatically defines the structures of interest may help to reduce this operator-dependent variability. In the sample analyzed, significant but negligible to low correlations were found between body weight and pituitary size and between age and pituitary height and length. Hence, the actual effect of these variables on the pituitary gland remains questionable. In addition, no differences between genders were found.

According to this study, a pituitary gland height > 4 mm or a P:B ratio > 0.49 mm should be considered enlarged. The reference ranges obtained from this study may help assess pituitary gland size in DSH cats with suspected neoplastic lesions affecting the pituitary gland, in surgical or radiation therapy planning, and in monitoring response to treatment.

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