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Association of Extreme Brachycephaly With Persistent Fontanelles in Adult Chihuahuas

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Keywords: computed tomography | cranial base | cranial index | craniometric measurement

ABSTRACT

Background: Although persistent fontanelles (PFs) are common in adult Chihuahuas, their association with cranial morphology remains unknown.

Objectives: To identify whether cranial morphology is associated with PFs in Chihuahuas and if bodyweight is associated with cranial morphology in this breed.

Animals: Fifty client-owned Chihuahuas.

Methods: In this retrospective cross-sectional study using computed tomography images, we measured two different cranial base lengths (1 and 2), cranial length, height, and width, and two craniofacial angles. We calculated the ratios of cranial height to cranial base lengths 1 and 2, cranial height to length, cranial height to width, and cranial width to length (cranial index [CrI]). We evaluated if total PF area and number of cranial sutures affected by PFs were associated with craniometric measurements and their ratios and craniofacial angles. Additionally, we evaluated if the craniometric ratios were associated with bodyweight.

Results: Total PF area was larger and number of cranial sutures affected by PFs higher in dogs with higher cranial height to cranial base length ratios 1 (estimate, [95% confidence interval], *p*: 2.295, [1.204–4.377], $p = 0.01$ and 1.720, [1.212–2.442], $p = 0.002$, respectively) and 2 (1.203, [1.069–1.354], $p = 0.003$ and 1.087, [1.011–1.169], $p = 0.02$, respectively) and CrI (1.225, [1.079–1.391], $p = 0.002$, and 1.134, [1.057–1.215], $p < 0.001$, respectively). Higher CrI was associated with lower bodyweight (−2.600, [−4.102 to −1.098], $p = 0.001$).

Conclusion and Clinical Importance: Our results suggest that in Chihuahuas, lower bodyweight is associated with more extreme brachycephaly and extreme brachycephaly is associated with PFs.

Abbreviations: CCJ, craniocervical junction; CFA, *Canis familiaris* autosome; CI, confidence interval; CM, Chiari-like malformation; CrI, cranial index; CSF, cerebrospinal fluid; CT, computed tomography; ICC, intraclass correlation coefficient; IGF-1, insulin-like growth factor 1; IGF-1R, insulin-like growth factor 1 receptor; MRI, magnetic resonance imaging; PF, persistent fontanelle; SM, syringomyelia; SMOC2, SPARC-related modular calcium binding protein 2.

1 | Introduction

Breed standards for Chihuahuas favor a low bodyweight and a brachycephalic, apple-shaped head [1]. Persistent fontanelles (PFs) are common in adult Chihuahuas and are associated with low bodyweight, syringomyelia (SM), ventriculomegaly, craniocervical junction (CCJ) overcrowding, and clinical signs related to Chiari-like malformation (CM) and SM [2, 3]. Although recent studies demonstrated the frequent occurrence and clinical relevance of PFs, their pathogenesis remains largely unknown. Brachycephalic dogs are predisposed to CM, SM, and ventriculomegaly, but it is currently unknown whether brachycephaly also predisposes to PFs [4–7].

Brachycephaly means "short head," and dogs with extremely short muzzle and short and wide head have traditionally been considered as brachycephalic [8, 9]. However, brachycephalic features are variable and in some dogs, the braincase can be extremely short and wide without a markedly shortened muzzle [10]. Additionally, brachycephalic dogs exhibit an increase in cranial height with rostrally doming frontal bones [7, 11]. This compensatory cranial growth in height is considered as the extended definition of brachycephaly and represents neotenic features that are favored in breeding of brachycephalic dogs [4, 6, 12]. Several morphological indexes have been used to divide dogs into brachycephalic, mesaticephalic, and dolichocephalic breeds [9, 10, 13]. For example, cranial index (CrI) describes the width to length ratio of the cranial vault, whereas cephalic index describes the width to length ratio of the entire head [9]. Higher values indicate more severe brachycephaly. However, these indexes are continuous variables, and no specific threshold values exist for brachycephaly in dogs.

It is currently believed that development of brachycephalic cranial shape in dogs is influenced by craniosynostosis, meaning premature fusion of the cranial sutures and premature closure of the cartilaginous junctions of the cranial base (the synchondroses) [14, 15]. Cranial growth occurs at the cranial sutures and synchondroses and is regulated by signals from dura mater and directed by the growing brain [16, 17]. Craniosynostosis prevents growth at the affected sutures, causing compensatory growth parallel to the patent sutures and resulting in abnormal cranial shape [18, 19].

Previous studies describing different dog breeds revealed a correlation between low bodyweight and increasing CrI and cephalic index [20, 21]. A possible explanation for this is that in small-sized dogs, breeding is often selective for brachycephalic head shape [21]. However, it is currently unknown if cranial morphology is associated with bodyweight in Chihuahuas.

The primary aim of this study was to investigate whether cranial morphology, more specifically extreme brachycephaly and shape of the frontal bones, is associated with larger and higher number of PFs in adult Chihuahuas. The secondary aim was to determine if bodyweight is associated with cranial morphology in Chihuahuas. Our hypotheses were that Chihuahuas with extreme brachycephaly and more rostrally bulging frontal bones have more and larger PFs. Additionally, we hypothesized that lower bodyweight in Chihuahuas is associated with extreme brachycephaly.

2 | Materials and Methods

This was a retrospective cross-sectional study examining the association of cranial morphology with total PF area and number of cranial sutures affected by PFs using computed tomography (CT) images.

2.1 | Selection of Animals

Data were collected from 50 client-owned adult Chihuahuas that were part of study cohort, which included 53 Chihuahuas with and without SM/CM-related clinical signs [2, 3, 22]. As inclusion criteria of the cohort, dogs with SM/CM-related clinical signs could be of any age, and they were included in the study if they had at least one clinical sign typical of SM or CM and the magnetic resonance imaging (MRI) of head and cervical spine did not reveal other causes for clinical signs except SM, CCJ overcrowding, ventriculomegaly, or combination thereof. Diagnostic imaging was part of a clinical work-up for dogs affected by SM/CM-related clinical signs. Dogs without clinical signs had to be at least 3 years of age and free of any signs of illness. For these dogs, diagnostic imaging was performed for pre-breeding screening of SM and CM. From the initial study cohort, we included all dogs that underwent CT for the present study. The bodyweight of the dogs was recorded at the time of the diagnostic imaging.

The dogs were recruited from the animal flow of Veterinary Teaching Hospital of University of Helsinki, Finland, between the years 2012 and 2015. The study was approved by the Finnish National Animal Experiment Board. Participation was voluntary and owners signed a consent form prior to participation.

2.2 | Diagnostic Imaging Procedure

CT images of head and cervical spine to the level of the third cervical vertebra were acquired using a helical dual-slice scanner (Somatom Emotion Duo, Siemens AG, Forchheim, Germany) with bone algorithm, 1.0-mm slice thickness, 2-mm feed/rotation, 0.5-mm reconstruction increment, and 110 kV. Dogs were under general anesthesia and positioned either in dorsal or ventral recumbency.

2.3 | Analysis of Persistent Fontanelles

Analysis of PFs of the dogs was performed similar to that described [2]. Briefly, the presence of PFs was first evaluated from three-dimensional volume-rendered CT images using OsiriX Medical Imaging Software (Pixmeo SARL, Bernex, Switzerland). A PF was defined as complete loss of bone at the cranial sutures observed on the dorsal, lateral, and caudal surfaces of the cranium. The total PF area and the number of cranial sutures affected by PFs were determined from multiplanar CT reconstructions independently by two board-certified veterinary neurologists unaware of the clinical status. The closed polygon tool of OsiriX was used to measure fontanelle area. A maximum intensity projection technique was applied to overcome the problem of large fontanelles extending over the convex surface of the cranium. A slice thickness of 14–16 mm was chosen for optimal visualization of the fontanelle to conduct the area measurement. In case of disagreement regarding the presence and number of the affected sutures, the assessors reassessed the images together to reach a consensus.

2.4 | Craniometric Analysis

CT images were viewed using OsiriX Medical Imaging Software with bone window settings (window level 500, window width 3500). Two assessors conducted the measurements independently from anonymized CT images. The first assessor (A.-M.K.) is a board-certified neurologist. The second assessor (E.R.) is an experienced veterinarian in radiology. Assessors were blinded to the total PF area, the number of affected cranial sutures, and to the results from the other assessor while performing the measurements.

2.4.1 | Craniometric Measurements

Several measurements were conducted from CT images to evaluate cranial morphology (Figure 1). Two different lengths of the cranial base, cranial length, and cranial height were measured from mid-sagittal images. Cranial width was measured from transverse images. Commonly recognized anatomic landmarks were used when appropriate (Table 1) [9]. The measurements were defined as follows:

1. Cranial base length 1: The length was measured from basion to rostral border of the presphenoid bone. The measurement was performed as a straight line along the ventral border of the cranial base even if the cranial base was convex in shape.
2. Cranial base length 2: The length was measured from basion to the caudoventral tip of the frontal bone, including the cribriform plate.
3. Cranial length: The length was measured from the nasion to the most caudal point of the cranium. If the nasofrontal sutures at the nasion were not visible, visual approximation of the most rostral point of the cranium was used. The caudal point of the cranium was defined as the most caudal edge of the cranium, which could be either inion or the more ventrally located caudal border of the supraoccipital bone.
4. Cranial height: The maximum height of the cranium perpendicular to the cranial base.
5. Cranial width: The maximum width of the cranium.

In case the cranium had large PFs complicating the determination of anatomic landmarks, an imaginary line representing the cranial surface was drawn to enable measuring.

The following ratios were calculated to determine the cranial shape:

1. Cranial height to cranial base length 1
2. Cranial height to cranial base length 2
3. Cranial height to length
4. Cranial height to width
5. Cranial index (CrI): cranial width × 100/cranial length.

2.4.2 | Craniofacial Angles

Two different angles (craniofacial angles 1 and 2) representing the rostral bulging of the frontal bones were measured from mid-sagittal images as described in Figure 2.

TABLE 1 | Anatomic landmarks used for craniometric measurements.

Anatomic landmark	Definition
Nasion	Junction of the left and right nasofrontal sutures in the midline
Inion	Most caudal edge of external occipital protuberance
Basion	Caudal border of the basioccipital bone

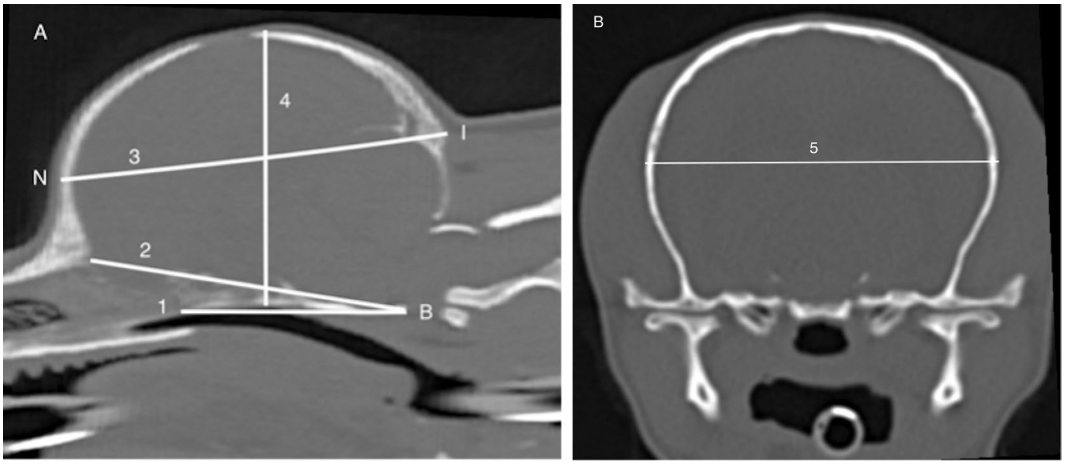

FIGURE 1 | Craniometric measurements and anatomic landmarks illustrated on mid-sagittal (A) and transverse (B) CT images of a Chihuahua cranium (window level 500, window width 3500). (1) cranial base length 1, (2) cranial base length 2, (3) cranial length, (4) cranial height, and (5) cranial width. B, basion; I, inion; N, nasion.

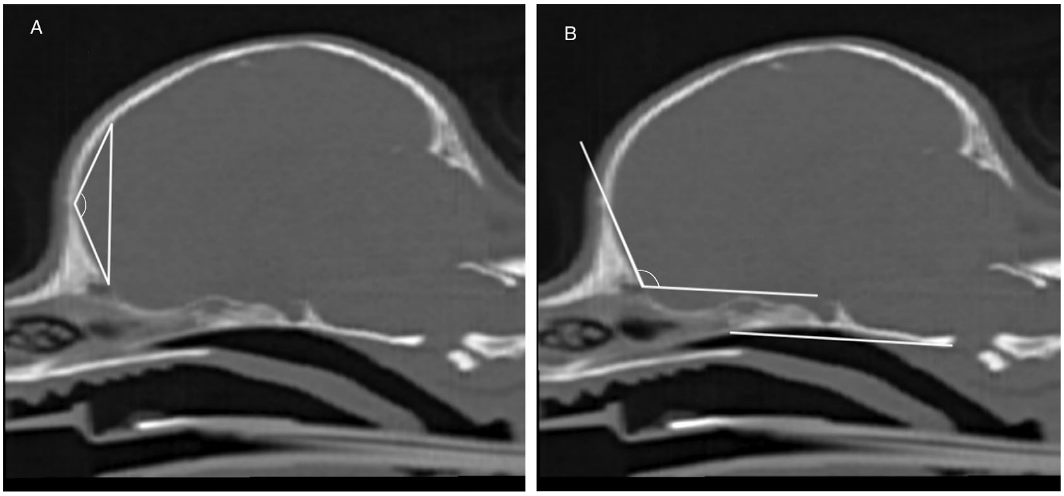

FIGURE 2 | Craniofacial angles 1 and 2 on mid-sagittal CT images of Chihuahua cranium (window level 500, window width 3500). (A) Angle 1; the angle between the lines drawn from the most rostral point of the cranium towards a vertical line of the rostral cranium positioned perpendicular to cranial base and drawn from the caudoventral tip of the frontal bone to the inner surface of the dorsal cranium. (B) Angle 2; the angle between the most rostral point of the cranium and a horizontal line parallel to cranial base extending to the caudoventral tip of the frontal bone.

2.5 | Statistical Analyses

2.5.1 | Inter- and Intra-Rater Reliability

To evaluate the repeatability of the measurement method, the inter- and intra-rater reliabilities were assessed for each parameter separately. Inter-rater reliability was evaluated in two different ways. First, a repeatability statistic between the assessors was calculated from a one-way analysis of variance model, where the effect of the dog served as the sole fixed effect. In this model, the within-group variation describes the variation between the assessors. These repeatability values can be considered as a percentage of perfect agreement. Second, Krippendorff's α with 95% confidence intervals (CI) were calculated to determine inter-rater reliability estimate between the two assessors. In this analysis, a value of 1 stands for perfect agreement, a value of 0.80 means similar interpretation, and a value of 0.67 describes the lowest acceptable limit [23].

To evaluate intra-rater reliability, craniometric measurements were repeated 3–6 months after the first measurements for 25 of 50 randomly selected dogs. Intraclass correlation coefficients (ICC) with 95% confidence intervals (95% CIs) were calculated to assess the consistency between the repeats within assessor (Table 4). Values < 0.5 indicate poor reliability, values between 0.5 and 0.75 moderate reliability, values between 0.75 and 0.9 good reliability, and values > 0.9 excellent reliability [24].

2.5.2 | Craniometric Measurements and Associated Ratios and Persistent Fontanelles

The mean value of the craniometric measurements from both assessors were used for calculating the ratios and for statistical analysis. Total PF area and total number of cranial sutures affected by PFs were used to investigate the association of PFs with craniometric measurements, including cranial base length 1 and 2, cranial length, cranial height, cranial width and the ratios of cranial height to cranial base lengths 1 and 2, cranial height to length, cranial height to width and cranial width to length (CrI), and craniofacial angles 1 and 2. Due to variation in body size of the dogs, individual measurements were complemented with the ratios of the cranial measurements in statistical analysis. As some dogs had several fontanelles at one cranial suture and the size of the fontanelles varied, the total number of cranial sutures affected by PFs was used instead of total number of fontanelles.

The association of total PF area and craniometric measurements were analyzed using univariate linear regression analysis. The total PF area was first log-transformed for analysis to satisfy the normality assumptions of the parametric statistical modeling. Because of some 0 observations (four dogs lacked a fontanelle), the transformation was conducted as log (total area + 1). The log-transformed total area served as the response and each craniometric measurement was assessed separately as the fixed term. The normality of the model residuals was investigated using Shapiro–Wilks tests and normal QQ-plots. Fit plots were generated for the statistically significant factors, including 95% confidence and prediction intervals. The association of total number of cranial sutures affected by PFs and craniometric measurements were analyzed using univariate Poisson regression; the total number of cranial sutures affected by PFs served as the response, with each of the 12 craniometric measurements assessed separately as the fixed term. The effects were quantified by calculating estimates and 95% CIs from the fitted models for 1/0.1/0.01 unit (depending on the measurement) increase in the corresponding craniometric measurement.

The association of craniometric measurements and bodyweight of the dogs was analyzed with linear regression analysis, where the ratios of the craniometric measurements (cranial height to cranial base length 1 and 2, cranial height to length, cranial height to width, and CrI) were each used as the response and the weight of the dog as the explaining factor for each measurement separately.

p values < 0.05 were considered statistically significant. All p values are two-sided and not adjusted for multiple testing. All statistical analyses were performed using the SAS System for Windows, version 9.4 (SAS Institute Inc., Cary, NC).

3 | Results

Fifty (27 females and 23 males) dogs were included in this study. The study included 26 smooth-haired and 23 long-haired Chihuahuas. One dog was a Chihuahua mix. Mean ± SD age of the dogs was 58 ± 28 months (range: 7–139 months) and mean ± SD weight was 2.8 ± 0.6 kg (range: 1.4–4.3 kg).

3.1 | Inter- and Intra-Rater Reliability

Inter-rater repeatability was ≥ 92% for measurement of cranial base lengths 1 and 2, cranial length, cranial height, and cranial width and ≥ 85% for craniofacial angles 1 and 2 (Table 2). Furthermore, Krippendorff's α values were > 0.80 for all measurements except for craniofacial angles 1 and 2, which were 0.780 for angle 1 (95% Cl: 0.654–0.964) and 0.713 for angle 2 (95% Cl: 0.603–0.808; Table 2). Intra-rater reliability was high, with ICC values > 0.80 except for craniofacial angle 2 (overall ICC value 0.745, 95% Cl: 0.593–0.845; Table 3).

TABLE 2 | Inter-rater repeatability and Krippendorff's α reliability estimates between the 2 assessors.

Variable	Inter-rater reliability	Krippendorff's α	95% CI
Cranial base length 1	92.080	0.843	0.748–0.921
Cranial base length 2	96.601	0.933	0.892–0.964
Cranial length	97.094	0.943	0.913–0.967
Cranial height	97.411	0.949	0.901–0.979
Cranial width	96.801	0.937	0.884–0.975
Angle 1	88.824	0.780	0.654–0.964
Angle 2	85.467	0.713	0.603–0.808

Note: Inter-rater reliability includes repeatability and Krippendorff's α estimates of the cranial base lengths 1 and 2, cranial length, cranial height, cranial width, and craniofacial angles 1 and 2 between the 2 assessors.
Abbreviation: CI, confidence interval.

3.2 | Craniometric Ratios

The mean ratios of the craniometric measurements are presented in Table 4. The mean ± SD CrI of the dogs was 82.24 ± 3.67 (range: 74.25–89.61).

3.3 | Craniometric Measurements and Associated Ratios and Persistent Fontanelles

Total PF area was significantly larger in dogs with shorter cranial base lengths 1 and 2 ($p = 0.01$ and $p = 0.006$, respectively). The number of cranial sutures affected by PFs was higher in dogs with shorter cranial base length 1 ($p = 0.006$; Table 5). Total PF area was significantly larger and number of cranial sutures affected by PFs was higher in dogs with higher ratio of cranial height to cranial base length 1 ($p = 0.01$ and $p = 0.002$, respectively) and 2 ($p = 0.003$ and $p = 0.02$, respectively; Table 5, Figures 3 and 4).

The number of cranial sutures affected by PFs was higher in dogs with increased cranial width ($p = 0.01$), indicating that dogs with wider cranium had more PFs (Table 5). Total PF area was not associated with cranial width ($p = 0.11$). The number of cranial sutures affected by PFs was significantly higher in dogs with lower cranial height to width ratio, indicating that dogs with wider cranium in relation to cranial height had more affected cranial sutures

TABLE 4 | Mean ratios of the craniometric measurements in Chihuahuas of this study.

Ratio	Mean	Standard deviation	Range
Cranial height: cranial base length 1	1.27	0.07	1.06–1.43
Cranial height: cranial base length 2	0.90	0.04	0.77–0.97
Cranial height: length	0.71	0.02	0.68–0.74
Cranial height: width	0.86	0.04	0.78–0.99
Cranial width × 100/ length (CrI)	82.24	3.67	74.25–89.61

Abbreviation: CrI, cranial index.

TABLE 3 | Intra-rater reliability: Intraclass correlation coefficients of the craniometric measurements.

Variable	Assessor 1 ICC	95% CI	Assessor 2 ICC	95% CI	Overall ICC	95% CI
Cranial base length 1	0.875	0.743–0.941	0.966	0.926–0.984	0.909	0.847–0.947
Cranial base length 2	0.969	0.932–0.986	0.981	0.959–0.991	0.973	0.954–0.985
Cranial length	0.952	0.955–0.985	0.994	0.986–0.997	0.974	0.955–0.985
Cranial height	0.938	0.868–0.971	0.990	0.978–0.996	0.963	0.936–0.978
Cranial width	0.938	0.868–0.971	0.996	0.991–0.998	0.965	0.940–0.980
Angle 1	0.820	0.642–0.914	0.899	0.790–0.953	0.848	0.749–0.910
Angle 2	0.702	0.442–0.853	0.812	0.627–0.910	0.745	0.593–0.845

Note: Intra-rater reliability includes intraclass correlation coefficients of cranial base lengths 1 and 2, cranial length, cranial height, cranial width, and craniofacial angles 1 and 2 to assess the repeatability of the measurements.
Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient.

($p = 0.01$; Table 5 and Figure 4). There was no significant association with the total PF area and cranial height to width ratio. Total PF area was significantly larger and the number of cranial sutures affected by PFs higher in dogs with higher CrI ($p = 0.002$ and $p < 0.001$, respectively; Table 5 and Figures 3 and 4).

3.4 | Craniofacial Angles and Persistent Fontanelles

Craniofacial angles were not associated with either the total PF area or number of cranial sutures affected by PFs (Table 5).

3.5 | Cranial Shape and Weight

Cranial height to width ratio was smaller in dogs with lower bodyweight ($p < 0.001$), indicating that smaller dogs had wider cranium in relation to cranial height. Cranial index was significantly higher in dogs with lower bodyweight ($p = 0.001$; Table 6).

4 | Discussion

We investigated the association of cranial morphology to PFs and bodyweight in adult Chihuahuas. Consistent with our hypotheses, higher CrI, indicative of greater brachycephaly, was associated with more and larger PFs. Furthermore, dogs more affected with PFs had a wider cranium, shorter cranial base, and increased cranial doming. Our findings did not support our hypothesis that rostrally bulging frontal bones would be associated with more and larger PFs. Finally, our data showed that lower bodyweight in Chihuahuas was associated with more extreme brachycephaly, supporting our hypothesis.

Cranial base length 1 was shorter in dogs with larger total PF area and higher number of cranial sutures affected by PFs. Furthermore, cranial base length 2, including the cribriform plate, was shorter in dogs with larger total PF area. When cranial height was compared with cranial base lengths 1 and 2, both ratios were higher in dogs with larger total PF area and higher number of cranial sutures affected with PFs. Longitudinal growth of the cranium occurs at the cranial base synchondrosis joining the presphenoid, basisphenoid, and basioccipital bones [9]. Premature closure of the cranial base synchondroses leads to shortening of the cranial base. This restricts longitudinal growth of the cranium and causes compensatory growth in height, resulting in cranial doming [18, 25]. This compensatory growth occurs in brachycephalic dogs and extremely brachycephalic peke-face Persian cats that have premature closure of the cranial base synchondroses, especially the spheno-occipital synchondroses [14, 15, 26]. Interestingly, our results showed that shortened cranial base and increased cranial doming were also associated with more PFs in Chihuahuas.

TABLE 5 | Association of total persistent fontanelle area and number of cranial sutures affected by persistent fontanelles with craniometric measurements.

	Association of total persistent fontanelle area and craniometric measurements (linear regression analysis)			Association of total number of cranial sutures affected by persistent fontanelles and craniometric measurements (univariate Poisson regression)		
Variable	**Estimate**	**95% CI**	***p***	**Estimate**	**95% CI**	***p***
Cranial base length 1	**0.748**	**0.597–0.937**	**0.01**	**0.852**	**0.761–0.954**	**0.006**
Cranial base length 2	**0.758**	**0.626–0.919**	**0.006**	0.899	0.808–1.001	0.05
Cranial length	0.843	0.658–1.079	0.17	0.968	0.850–1.104	0.63
Cranial height	0.992	0.735–1.340	0.96	1.011	0.858–1.191	0.89
Cranial width	1.175	0.965–1.432	0.11	**1.135**	**1.030–1.252**	**0.01**
Cranial height: cranial base length 1	**2.295**	**1.204–4.377**	**0.01**	**1.720**	**1.212–2.442**	**0.002**
Cranial height: cranial base length 2	**1.203**	**1.069–1.354**	**0.003**	**1.087**	**1.011–1.169**	**0.02**
Cranial height: length	1.293	0.960–1.743	0.09	1.012	0.859–1.193	0.88
Cranial height: width	0.886	0.775–1.012	0.07	**0.908**	**0.842–0.979**	**0.01**
Cranial width × 100/length (CrI)	**1.225**	**1.079–1.391**	**0.002**	**1.134**	**1.057–1.215**	**< 0.001**
Angle 1	0.959	0.883–1.040	0.30	0.965	0.923–1.009	0.12
Angle 2	1.056	0.947–1.177	0.32	1.048	0.988–1.111	0.12

Note: $p < 0.05$ were considered statistically significant. Variables with a p-value < 0.05 are bolded.
Abbreviations: CI, confidence interval; CrI, cranial index.

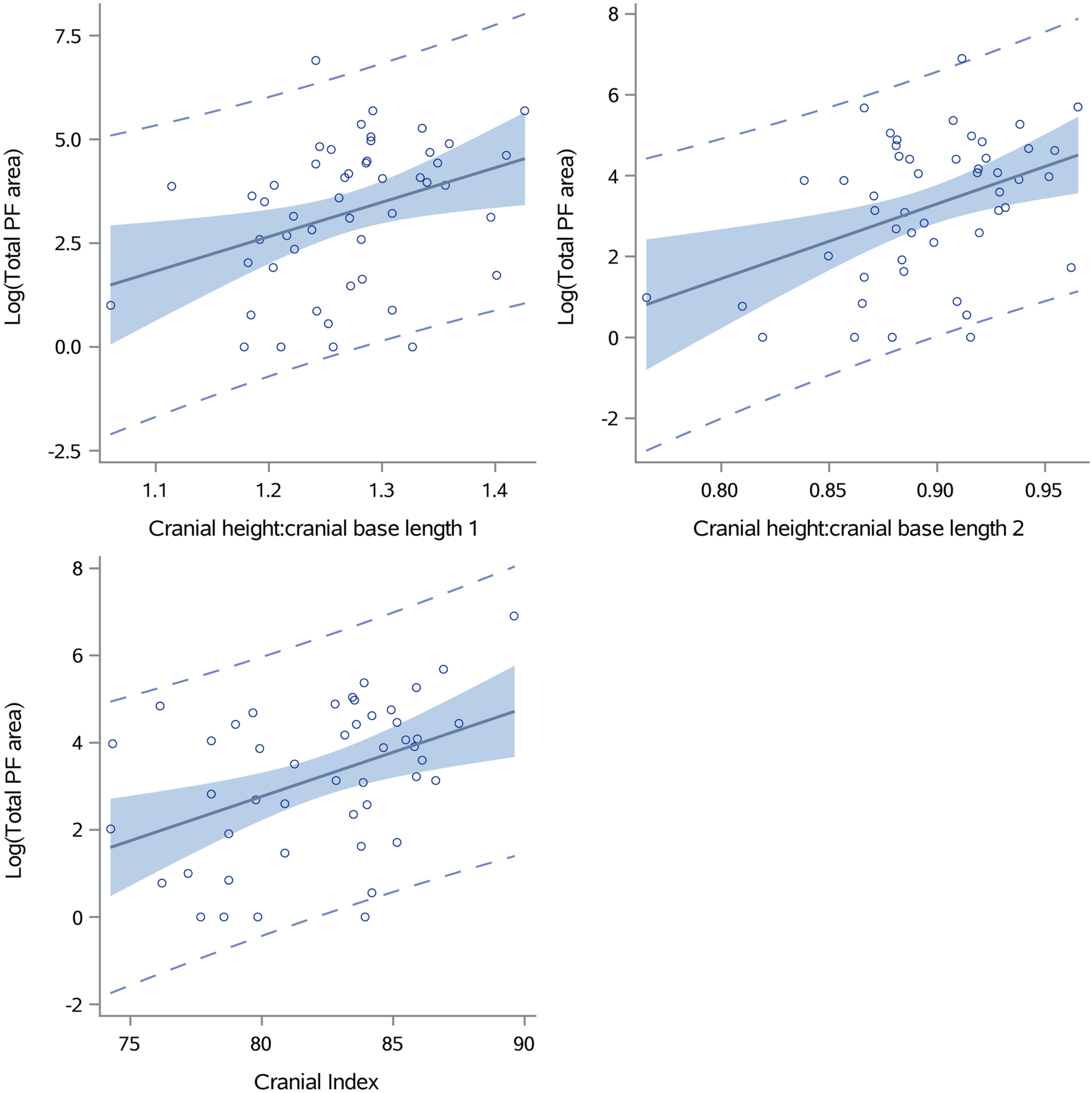

FIGURE 3 | Fit plots together with 95% confidence (shaded area) and prediction (dashed lines) intervals for linear regression analysis demonstrating the association of log-transformed total persistent fontanelle area with significant craniometric ratios including ratios of cranial height to cranial base length 1 and cranial height to cranial base length 2 and cranial index.

Shortened cranial base could disturb CSF flow at CCJ and absorption of CSF through cranial base and olfactory lymphatics at the cribriform plate, which might predispose to PFs in several ways. First, shortening of the cranial base is associated with increased proximity of the basioccipital bone to the atlas and axis contributing to CCJ overcrowding observed as Chiari-like malformation, atlanto-occipital overlapping, medullary elevation, and dorsal spinal cord compression [6, 22, 27]. Overcrowding of the CCJ disturbs CSF flow and predisposes to SM and enlargement of the lateral ventricles [6, 28–30]. Second, decreased size of the rostral cranial fossa, including the cribriform plate, in brachycephalic dogs might cause overcrowding of the brain tissue in this area and disrupt CSF absorbance at the cribriform plate [31, 32]. In many mammals, in addition to cranial base lymphatics, the cribriform plate serves as an important site of CSF drainage into the nasal mucosal lymphatics [33–37]. Disrupted CSF absorption at the cribriform plate might contribute to development of elevated intracranial pressure, ventriculomegaly, and hydrocephalus as observed in sheep and rat models [34, 38]. Finally, shortening of the cranial base might predispose to stenosis of the jugular foramina, which might compromise venous outflow from the cranium and cause venous hypertension [39, 40]. This could increase intracranial pressure and further impair CSF flow as suggested in hydrocephalic children with

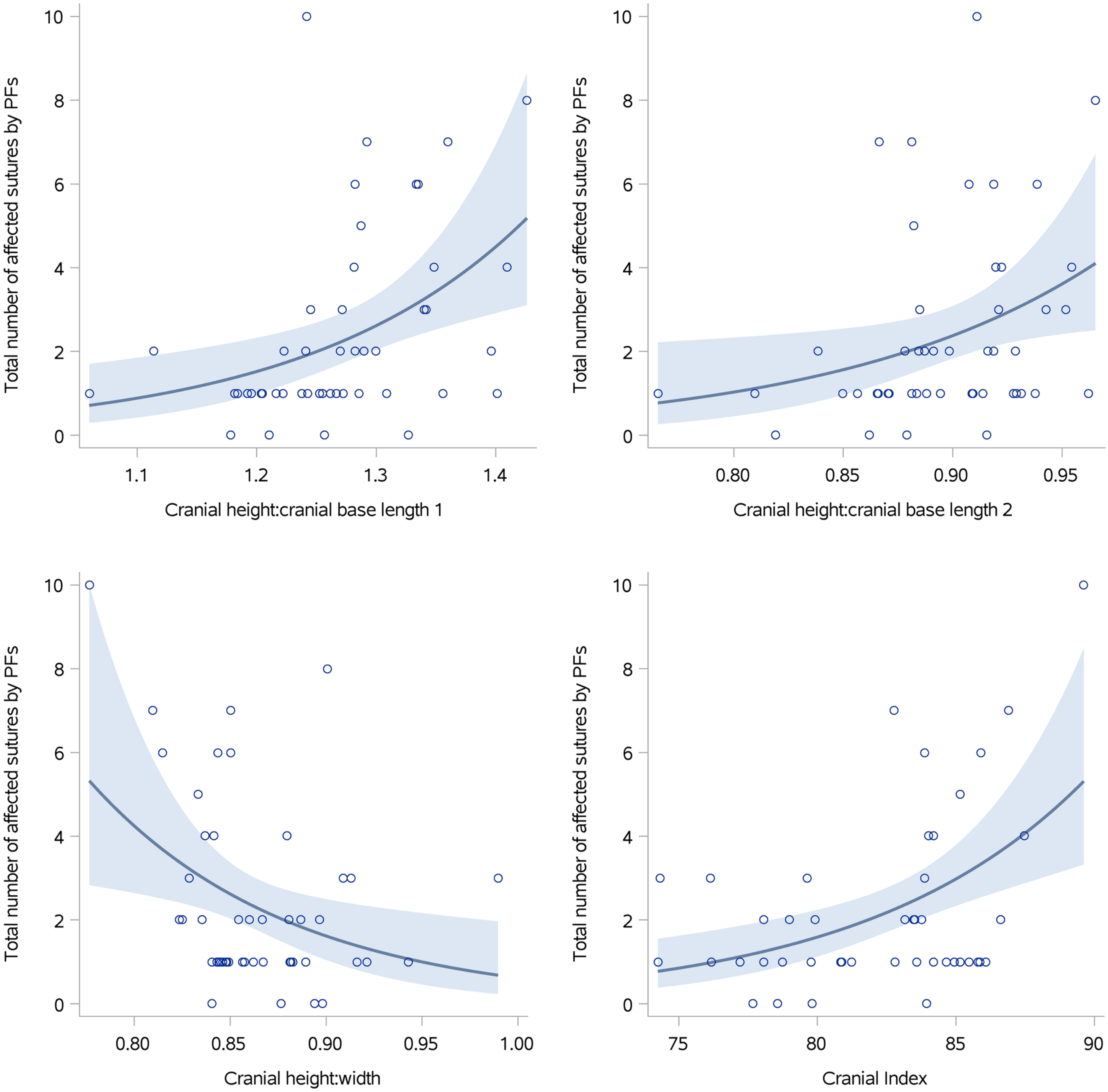

FIGURE 4 | Fit plots together with 95% confidence (shaded area) intervals for Poisson regression demonstrating the association of total number of cranial sutures affected by PFs with significant craniometric ratios including ratios of cranial height to cranial base length 1, cranial height to cranial base length 2, and cranial height to width and cranial index.

TABLE 6 | Linear regression analyses for the association of craniometric ratios and bodyweight.

Variable	Effect	Estimate	95% CI	*p*
Cranial height: cranial base length 1	Weight (1 kg)	−0.021	−0.054–0.012	0.20
Cranial height: cranial base length 2	Weight (1 kg)	−0.015	−0.033–0.003	0.10
Cranial height: length	Weight (1 kg)	−0.000	−0.008–0.007	0.90
Cranial height: width	Weight (1 kg)	**0.027**	**0.012–0.042**	**< 0.001**
Cranial width × 100/length (CrI)	Weight (1 kg)	**−2.600**	**−4.102 to −1.098**	**0.001**

Note: $p < 0.05$ were considered statistically significant. Variables with a p-value < 0.05 are bolded.
Abbreviations: CI, confidence interval; CrI, cranial index.

concurrent jugular foramina stenosis [41]. Jugular foramina stenosis also occurs in Cavalier King Charles Spaniels with SM, possibly causing venous hypertension and contributing to development of SM in these dogs [42]. Extreme brachycephaly requires compensatory mechanisms to accommodate the brain in the cranial vault. Possible ventriculomegaly and increase in venous pressure might further increase overall strain exerted on the meninges. Distension of the meninges could cause remodeling of the existing cranial bone and disturb ossification of the developing bone and hence affect formation of PFs in Chihuahuas.

Although cranial base lengths 1 and 2 were associated with PFs, the entire cranial length was not associated with total PF area or number of cranial sutures affected by PFs. One explanation might be that rostral bulging of the frontal bones compensates for restricted longitudinal growth of the cranial base. Such an effect occurs in children with achondroplasia. In these children, the cranial base is shortened without change in overall cranial length [43].

Increased cranial width was associated with a higher number of cranial sutures affected by PFs. Furthermore, the cranial height to width ratio was lower in dogs with a higher number of cranial sutures affected by PFs. These findings suggest widening of the cranium in dogs with more PFs. In brachycephaly, widening of the cranium compensates for restricted longitudinal growth and might be associated with craniosynostosis of the cranial sutures [26, 44]. In children, brachycephalic cranial shape is associated with bilateral coronal (frontoparietal) craniosynostosis [18, 44]. Similarly, bilateral coronal craniosynostosis occurs in extremely brachycephalic peke-face Persian cats with wide, short, dorsally round, and dome-shaped cranium. Peke-face Persian cats also have osseous defects of the frontal and parietal bones, and these defects are significantly associated with peke-face morphology [26, 45]. Studies evaluating closure of the coronal sutures in dogs are lacking. However, brachycephalic dogs have more other closed or closing cranial sutures than mesaticephalic and dolichocephalic dogs [15]. Accordingly, cranial suture closure and occurrence of PFs in young growing Chihuahuas should be investigated to evaluate possible association of craniosynostosis with PFs.

Higher CrI was associated with larger total PF area and higher number of affected cranial sutures by PFs, indicating widening and shortening of the cranium in dogs with larger and more numerous PFs. Although there was substantial variation in CrI values in the dogs of this study, the mean CrI (82.24) of this study population indicates brachycephalic cranial morphology. Furthermore, the dogs with the highest values can be considered as having extreme brachycephaly. As a comparison, in a group of Cavalier King Charles Spaniels, a mean CrI of 74.76 was reported, while the mean CrI of other brachycephalic dogs of the study were 66.74 [10]. A CrI value of 84 has been reported for Pekingese and Griffon Bruxellois dogs, both defined as brachycephalic breeds [46]. Brachycephalic cranial shape causes reorganization of the brain tissue, which might result in overcrowding of the cranial vault and hence affect formation of PFs in Chihuahuas [20, 47, 48]. In dogs, higher CrI is associated with cerebellar indentation and impaction in the caudal cranial fossa [49]. Furthermore, size of the lateral ventricles increases with increasing CrI [21]. A higher cephalic index, describing the length and width of the entire head, is associated with increased ventral rotation of the longitudinal cerebral axis, ventrally positioned olfactory bulb, and SM, thus also indicating reorganization of the brain tissue [5, 20, 47].

Chihuahuas with short, wide, and dorsally doming cranium (meaning overall round cranial shape) had larger and more numerous PFs. In Chihuahuas, this type of round cranial shape is referred to as “apple-head” and is a desired feature in the breed [1]. In Chihuahuas, PFs are associated with SM/CM-related clinical signs [2] and hence it seems possible that breeding a round apple-shaped head predisposes Chihuahuas to these clinical signs. Similarly, short and dorsally doming cranium is associated with CM and SM in Cavalier King Charles Spaniels and Griffon Bruxellois dogs and also with CM-related pain in Cavalier King Charles Spaniels [4, 5, 7, 12].

Craniofacial angles 1 and 2 were not associated with total PF area or number of affected cranial sutures by PFs. In Chihuahuas, the shape of the rostral cranium is one of the features defined in breed standards recommending “forehead” that is rounded above the base of the muzzle [1]. This rostral bulging of the cranium, referred to as frontal bossing, is a typical feature in brachycephalic dogs and cats [5, 45]. Frontal bossing has also been observed in children with craniosynostosis [50]. However, our findings did not support our hypothesis that rostral bulging of the frontal bones is associated with PFs in Chihuahuas. The reason for this might be that measuring craniofacial angles from a two-dimensional image does not represent accurately enough the three-dimensional shape of the rostral cranium. Hence, further studies using three-dimensional measurement of the cranial shape might be necessary to more accurately evaluate the possible association of rostral bulging of the frontal bones and PFs in Chihuahuas.

Cranial height to width ratio was smaller in dogs with lower bodyweight, indicating that lower bodyweight was associated with a proportionally wider cranium. Furthermore, CrI was higher in dogs with lower bodyweight, indicating increased widening and shortening of the cranium in smaller dogs. Our results show that even within a single breed, cranial morphology can vary in relation to bodyweight. Similarly, small-sized dogs of different breeds have higher CrI when compared with larger dogs [21]. In domestic dogs, variation in bodyweight is large, with the Chihuahua being one of the smallest dog breeds in the world. Marked reduction in body size compared with a larger ancestor of the species is referred as miniaturization [51]. In general, species with lower bodyweight have a larger relative brain size than larger species, possibly suggesting limitation in neural tissue reduction during miniaturization to maintain essential functions of the nervous system [52–54]. Larger relative brain size might lead to changes in cranial morphology. In miniaturized hummingbirds and salamanders, the cranium is wider and more spherical in shape when compared with larger species, possibly because a spherical brain case can accommodate neural tissue compactly [53, 54]. In miniaturized hummingbirds, a more circular brain case is also associated with reduced cranial ossification, potentially allowing additional space for the brain as the reduced ossification first affects the inner surface of the cranial bone [54].

Miniaturization occurs because of natural evolution. In dogs, reduction in body size is due to human-made breeding selections. A mismatch between volume of brain parenchyma and caudal cranial fossa has been suggested in Cavalier King Charles Spaniels [55, 56]. One possible explanation is that the decrease in brain mass is inadequate compared with cranial volume during the miniaturization process in Cavalier King Charles Spaniels [56]. In Chihuahuas, widening of the cranium associated with decreasing bodyweight might reflect a means to accommodate a relatively large brain into a diminished cranial vault.

In dogs, both body size and head shape are influenced by the combined effects of several different genes, thus representing polygenic traits [57–59]. Several genetic variants are associated with body size and insulin-like growth factor 1 (*IGF-1*), and insulin-like growth factor 1 receptor (*IGF-1R*) genes appear to be particularly important regulators of small size in dogs [60–62]. Additionally, head shape is associated with several quantitative trait loci including loci in chromosomes CFA1, CFA5, CFA26, CFA32, and CFX [57]. The brachycephalic phenotype is suggested to be strongly associated with CFA1 [63], and a mutant variant of SPARC-related modular calcium binding (*SMOC2*) gene on the CFA1 is associated with brachycephaly explaining 36% of facial-length variation in the dogs of the study [59]. Interestingly, some of the genetic loci are associated with the cranial morphology as well as the body size [59]. Strong selection of phenotypic traits according to breeding guidelines affects the genotypes of the dogs. In Chihuahuas, the genetic basis of PFs remains unknown. It might be possible that selecting for low bodyweight and round head shape increases the occurrence of PFs in Chihuahuas.

Finally, when assessing inter-rater repeatability of the craniometric measurements, the percentage of agreement was high and Krippendorff's α showed similar interpretation for all measurements except for craniofacial angles, in which the agreement was slightly lower but still within acceptable limits [23]. The intra-rater reliabilities of the craniometric measurements were good to excellent [24] for cranial base lengths 1 and 2, cranial length, cranial height, cranial width, and craniofacial angle 1 and were moderate for craniofacial angle 2. These results indicate that the craniometric measurements were repeatable between the assessors and within the same assessor, thus increasing the validity of our findings.

There are some limitations in this study. While measuring cranial length, in some cases we had to use the most caudal point of the cranium, rather than the occipital protuberance, which was underdeveloped. We used visual approximation when evaluating the most rostral point of the cranium, as the nasofrontal sutures were poorly visible in some cases. Although we were unable to use standard landmarks for cranial length, this modified measurement was repeatable. As cribriform plate conformation can be variable in dogs with different cranial shape, the cranial base length 2 measurement from a two-dimensional mid-sagittal image might not represent the overall size of the three-dimensional cribriform plate [64]. However, the main interest of this study was to evaluate cranial base lengths in two different ways and hence we chose the mid-sagittal measurement, which represents the length of the cribriform plate in addition to cranial base bones. Finally, the body condition score of the dogs was not recorded and might have been over- or underweight in some dogs. As obesity is fairly common in Chihuahuas [65], the risk of overweight seems more likely and this might have underestimated the effect of bodyweight on cranial shape in our study.

5 | Conclusion

Extreme brachycephaly was associated with more and larger PFs in Chihuahuas. Furthermore, Chihuahuas with lower bodyweight had more extreme brachycephaly. As CrI was associated with low bodyweight and more numerous and larger PFs, it might be that low bodyweight and extreme brachycephaly are both required for formation of PFs in Chihuahuas.

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Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

The Finnish Animal Experimental Board (ESAVI/5794/04.10.03/2011 and ESAVI/9184/04.10.07/2014) approved obtaining CT images for diagnostic purposes. Authors declare human ethics approval was not needed for this study.

Conflicts of Interest

The authors declare no conflicts of interest.

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