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- 1 Title: Computed tomographic morphometry of tympanic bulla shape and position in brachycephalic
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- 14 Keywords: Brachycephalic, tympanic bulla location, middle ear effusion
- 15
- 16 Running Head: Tympanic Bulla Morphology in Brachycephalics

## 17 Abstract:

18 Anatomic variations in skull morphology have been previously described for brachycephalic dogs; however 19 there is little published information on interbreed variations in tympanic bulla morphology. This retrospective 20 observational study aimed to (1) provide detailed descriptions of the computed tomographic (CT) morphology 21 of tympanic bullae in a sample of dogs representing four brachycephalic breeds (Pugs, French Bulldogs, 22 English Bulldog, and Cavalier King Charles Spaniels) versus two mesaticephalic breeds (Labrador retrievers 23 and Jack Russell Terriers); and (2) test associations between tympanic bulla morphology and presence of 24 middle ear effusion. Archived head CT scans for the above dog breeds were retrieved and a single observer 25 measured tympanic bulla shape (width:height ratio), wall thickness, position relative to the 26 temporomandibular joint, and relative volume (volume:body weight ratio). A total of 127 dogs were sampled. 27 Cavalier King Charles Spaniels had significantly flatter tympanic bullae (greater width:height ratios) versus 28 Pugs, English Bulldogs, Labrador retrievers, and Jack Russell terriers. French Bulldogs and Pugs had 29 significantly more overlap between tympanic bullae and temporomandibular joints versus other breeds. All 30 brachycephalic breeds had significantly lower tympanic bulla volume:weight ratios versus Labrador 31 retrievers. Soft tissue attenuating material (middle ear effusion) was present in the middle ear of 48/100 (48%) 32 of brachycephalic breeds, but no significant association was found between tympanic bulla CT measurements 33 and presence of this material. Findings indicated that there are significant interbreed variations in tympanic 34 bulla morphology, however no significant relationship between tympanic bulla morphology and presence of 35 middle ear effusion could be identified.

### 36 Introduction:

37 Brachycephalic dogs have become increasingly popular and frequently present with brachycephaly related 38 abnormalities. The complex group of anatomical anomalies seen in these breeds has been associated with 39 congenitally shortened skull bones resulting in a relative hypertrophy of soft tissue structures within the head. 40 The increasing use of cross-sectional diagnostic imaging during the diagnostic evaluation of brachycephalic 41 dogs has identified an expanded list of abnormalities from the traditional stenotic nares, elongated soft palate 42 and tracheal hypoplasia. These include abnormalities of the nasal turbinates, increased mucosal contact points, 43 miniscule or absent frontal sinuses, ventral orientation of the olfactory bulb (cranio-facial angle), thickening of the soft palate and gastrooesophageal disease.<sup>1-7</sup> Middle ear effusion has also been reported to be a 44 45 common finding in cross-sectional imaging studies of the head in brachycephalic dog breeds.<sup>2, 8, 9</sup>

46

The authors have commonly observed middle ear effusion and interbreed variations in size, shape, and position of the tympanic bullae and thickness of the bulla wall on computed tomography (CT) examinations of brachycephalic dogs at our hospital. Aims of the current study were to provide more detailed descriptions of these morphologic variations in groups of dogs representing brachycephalic and mesaticephalic breeds; and to test hypotheses that morphologic characteristics will differ between breeds and that tympanic bulla morphology is associated with presence of middle ear effusion.

53

#### 54 Methods:

55 Dogs

56 The study was a retrospective, observational design. A first year surgery resident (B.M.) searched the 57 electronic medical records database of the author's hospital between July 2008–August 2015. Brachycephalic 58 dogs were considered for inclusion if they were pure-bred, greater than 1 year of age at the time of the study 59 and had CT scans of the head. Four major brachycephalic breeds were identified during the initial review and 60 cases were then collected until there was an even number of dogs in each group. Mesaticephalic dogs were 61 selected for inclusion if they were pure-bred Labrador retrievers or Jack Russell terriers, had CT scans of the 62 head, and had no evidence of head trauma, clinical ear disease, or aural neoplasia. Patient history and clinical 63 findings were recorded for all dogs meeting these initial inclusion criteria.

### 64 Computed tomographic scanning techniques

65 As part of the inclusion criteria, all CT scans had been acquired using a standardized protocol. The same 16-66 slice scanner was used (Mx8000 IDT, Philips, Best, The Netherlands), with all dogs placed under anesthesia 67 or sedation and positioned in sternal recumbency. Acquisitions extended from the nasal planum through the 68 first cervical vertebra. Combinations of axial and helical acquisitions were performed at the discretion of the 69 supervising clinician and depended on the nature of the original presenting complaint. Axial acquisitions used 70  $16 \times 0.75$  mm collimation, 0.75 mm reconstruction slice thickness with no overlap, pitch 1, 0.75 s rotation 71 time, 250 mA (nominal), 120 kVp, 500 mm acquisition field of view, with reconstruction field of view 72 dependent on patient body size (varying between 200 to 300 mm), and 768 × 768 matrix. Helical acquisitions 73 used  $16 \times 1.5$  mm collimation, 2 mm reconstruction slice thickness with 1.5 mm overlap, pitch 1, 0.5 s 74 rotation time, 200 mA (nominal) 120 kVp, 500 mm acquisition field of view, with reconstruction field of view 75 dependent on patient body size (varying between 200 to 300 mm),  $768 \times 768$  matrix. Reconstruction 76 algorithms used included high-frequency bone reconstruction and medium frequency soft-tissue 77 reconstruction algorithms. The CT scanner was calibrated weekly using the manufacturer's quality control

78 software.

#### 79 *Qualitative computed tomography evaluation techniques*

80 For dogs meeting the initial inclusion criteria, archived CT images were retrieved from the hospital's picture 81 archiving and communications system (PACS) and evaluated by a first year resident (B.M.) after consultation 82 with a board-certified veterinary radiologist (R.L.). Images were reviewed on a computer workstation (iMac, 83 27 Inch Monitor, Apple, CA) using commercially available DICOM image viewing software (OsiriX 64-bit, 84 version 6.1, Pixmeo, Switzerland). Dogs were excluded if CT studies had evidence of excessive motion 85 artifact or incomplete coverage of the anatomical regions of interest (ROI), or if images were reconstructed 86 using only medium frequency (mediastinum) or low-frequency (soft tissue or brain) algorithms. For dogs 87 meeting final inclusion criteria, presence or absence of middle ear effusion was recorded. Presence of middle 88 ear effusion was based on detection of soft tissue attenuating material within the tympanic bulla in 89 noncontrast-enhanced CT images. There was no additional evaluation of contrast-enhanced CT images to 90 distinguish between fluid and soft tissue.

92	Quantitative	computed	tomography	evaluation	techniques

93	All measurements were performed by a single observer with measurements recorded once. The CT
94	examinations were reviewed with a wide window and with bone reconstruction algorithms to ensure uniform
95	identification of tympanic bulla structures. Measurements were repeated for both the left and right tympanic
96	bullae. To adjust for variations in the orientation and position of structures of interest between individuals, all
97	measurements were performed from multiplanar reconstructions of CT images. The long-axis sagittal
98	reconstructions were oriented parallel to the line measured from the prosthion to the basion (the skull base).
99	The short axis, transverse reconstructions were oriented perpendicular to this line.
100	
101	Skull morphology was measured using the following criteria, based on those described previously by Hussein
102	et al., and were obtained using transverse and sagittal multiplanar reconstructions images <sup>3</sup> :
103	• Skull Width (SW) was defined as the widest width between the lateral margin of the left and right
104	zygomatic arches
105	• Skull Length (SL) was defined as the distance from the prosthion (the most rostral point of the
106	interincisive suture of the alveolar process of the maxilla) to the inion (most caudal midline point of
107	the external occipital protuberance of the occipital bone)
108	Tympanic bulla width and height measurements were performed using the following criteria. All
109	measurements were made from transverse multiplanar reconstructions images, at the level of the external
110	acoustic meatus with the greatest diameter determined from serial examinations of slices (Figure 1):
111	• Internal width of the tympanic bulla (WTB1) was defined as the maximal internal width of the
112	tympanic bullae at the level of the tympanic membrane, from the inner bony meatus, taken 90 degrees
113	to the height measurement.
114	• External width of the tympanic bulla (WTB2) was defined as the maximal external width of the
115	tympanic bulla measured as for the WTB1, to the external surface.
116	• Height of the tympanic bullae (HTB) was defined as the maximal internal height of the tympanic
117	bullae, taken 90 degrees to the width measurement.

- Transverse CT images were used to calculate the tympanic bulla volume (TBV). The internal
- circumference of the tympanic bulla was segmented freehand using a cursor for both left and right
- 120 bulla. The viewing software subsequently calculated the total internal bulla volume (cm<sup>3</sup>)
- 121 The following formulas were used to calculate relative values for statistical comparisons:
- 122

• Tympanic bulla internal width: height ratio (WHR Internal) = (WTB1 x 100) / HTB

123

٠

Tympanic bulla thickness = WTB1 - WTB2

• TBV:weight ratio = TBV/patient weight (cm<sup>3</sup>/kg)

125 The position of the tympanic bulla relative to the medial aspect of the temporo-mandibular joint was 126 calculated to determine if there was overlap of these structures. Overlap represents a relative rostral location 127 of the tympanic bulla relative to the temporomandibular joint. Measurements were obtained parallel to the 128 long-axis of the skull (Figure 2) from the prosthion to the; rostral internal tympanic bulla (PRTB), caudal 129 internal tympanic bulla (PCTB) and the medial temporo-mandibular joint (PTMJ). Relative location was 130 calculated to compensate for variation in size among the breed types used in this study, by computing indices 131 for the above parameters in analogy with the previous reported technique where; skull index = (skull width 132 (SW) x 100 / skull length (SL)).<sup>3</sup> The following indices were calculated: Rostral Tympanic Bulla Location 133  $(RTB) = (PRTB \times 100)/SL$ , Caudal Tympanic Bulla Location  $(CTB) = (PCTB \times 100)/SL$ , 134 Temporomandibular joint location (TMJ) = (PTMJ X 100)/SL. The relative overlap of the TMJ and tympanic 135 bulla were determined by the following formula (Overlap = RTB – TMJ), where a negative value represents

- 136 overlap of the two structures.
- 137

Measurements were repeated for both the left and right tympanic bullae. Animals were classified as having soft tissue attenuation within the tympanic bulla present unilaterally or bilaterally. A distinction between fluid and soft tissue by sampling and evaluation of contrast-enhancement was not part of this study.

141

# 142 Statistical Analysis:

143 Statistical analyses were performed using a statistical software package (SPSS Stat, Version 22, IBM Corp)

144 by one author (BM). Age and body weight data for all dogs in the study population is presented as mean and

145 range. Normality of data was assessed by Shapiro-Wilk's test (p<0.05). Width height ratio, tympanic bulla 146 thickness, Rostral tympanic bulla location, Caudal tympanic bulla location, temporomandibular joint location 147 and overlap, and TBV:weight ratios were compared between all breeds by one-way ANOVA. Post-hoc 148 analysis based on homogeneity of variance (Tukey-Kramer and Games-Howell) was used to identify 149 variations in anatomy between brachycephalic breeds and mesaticephalic breeds. These results are presented 150 as mean +/- standard deviation with 95% confidence intervals. Independent sample t-test were used to identify 151 variation in the mean thickness of brachycephalic breeds with and without soft tissue attenuating material 152 within the bulla. Values were considered significant at p < 0.05 for all tests.

153

#### 154 **Results:**

Age, sex, and weight distribution data for sampled dogs are presented in Table 1. A total of 100
brachycephalic dogs met the final inclusion criteria. All brachycephalic dogs were presented for further
evaluation of brachycephalic obstructive airway syndrome (BOAS). Breeds included 25 each of Pug, English
Bulldog, French Bulldog and Cavalier King Charles Spaniel. A total of 23 metacephalic dogs were included,
with 13 Labrador retrievers and 10 Jack Russell Terriers. Metacephalic dogs were presented for further
investigation of respiratory disease (12/23), neurological disease (5/23) mandibular neoplasia (4/23), and as
part of endocrine disease investigation (2/23).

162

163 Calculated ratios based on measurements of skull width and length (skull ratio) are also presented in Table 1. 164 Internal dimensions (width and height) of the tympanic bulla are reported in Table 2. Tympanic bulla wall 165 thickness (mm), tympanic bulla:temporomandibular joint overlap measurements (% skull length) and presence 166 of effusion are reported in Table 2 as well. The width-height ratio (Internal) for Cavalier King Charles 167 Spaniels was significantly different from that of all other breeds, reflecting a flatter bulla shape (Fig. 3 and 168 Table 2). French Bulldogs and English Bulldogs had significantly thicker tympanic bulla walls ( $2.5 \pm 1.3$  mm 169 and  $1.9 \pm 0.7$  mm, respectively) than those of all other breeds. Pugs, French Bulldogs, and English Bulldogs 170 had smaller tympanic bulla volume:weight ratios compared to those of all other breeds (Table 2). A 171 significantly more rostral location of the tympanic bulla with more overlap of the temporomandibular joint 172 and tympanic bulla was found in both Pugs and French Bulldogs, with an overlap of  $-1.15 \pm 2.21$  (95% CI

173 -2.06 to -0.23) and -0.5 +/- 2.3 (95% CI -1.45 to 0.45), respectively. This finding is illustrated in Figure 4
174 and numerically summarized in Table 2.

175

Soft tissue attenuating material was present in the middle ear of 48/102 (47.1%) of the brachycephalic dogs
(presented in Table 2) and none of the control dogs. Effusion was not significantly related to any of the
aforementioned parameters.

179

# 180 **Discussion:**

181 This study describes the morphology of the tympanic bulla in brachycephalic dogs, highlighting the variation 182 in shape, size/volume and position. Of particular note is the dorso-ventral flattening of the tympanic bulla in 183 Cavalier King Charles Spaniel, the increased thickness of the bulla wall of French Bulldogs and English 184 Bulldogs and the increasingly rostral location of the tympanic bulla with increasing skull index, most 185 noticeable in the French Bulldog and the Pug (Figure 4). These findings suggest that the tympanic bulla 186 characteristics, here collectively referred to as tympanic bulla malformation, are closely associated with the 187 brachycephalic phenotype. In analogy with children with craniofacial distortions, this tympanic bulla 188 malformation possibly develops due to early fusion of ventral skull base articulation.<sup>10</sup>

189

190 Cavalier King Charles Spaniel represent a unique subset of brachycephalic animals as they were found to have 191 significantly flatter tympanic bullae (defined by width:height ratios) than other brachycephalic breeds. There 192 was a high percentage of Cavalier King Charles Spaniel with soft tissue attenuating material in the middle ear 193 (68%), which may have falsely reduced the internal measurements obtained, increasing width:height ratio 194 (internal) artifactually<sup>11</sup>. However, given the lower bound of the 95% confidence interval measurements for 195 Cavalier King Charles Spaniel (width:height ratio(internal) 1.47 - 1.65)) was greater than the upper bound for 196 all breeds (except the French Bulldog which also had a high percentage of material in middle ear (80%) 197 (Table 1 and 2)), authors believe it is likely that these measurements represented real changes present in this 198 breed. Cavalier King Charles Spaniels have been described to have a unique disease resulting in the formation 199 of a buildup of highly viscous mucus within the middle ear (primary secretory otitis media or otitis media with effusion) of dogs without clinical evidence of otitis externa.<sup>10,12</sup> It is thought that, due to a lack of 200

201 inflammation or signs of infection in the middle ear, the disease is due to auditory tube dysfunction based on 202 possible anatomical changes of the middle ear or the auditory tube.<sup>8</sup> The finding of an anatomical variation in 203 the shape of the tympanic bulla of Cavalier King Charles Spaniel (Table 2 and Figure 4) may offer a potential 204 explanation for the pathogenesis of this disease in addition to the previously suggested changes in the 205 orientation and function of the auditory tube. However, given the lack of histopathology and contrast studies 206 in the current study, this hypothesis is speculative. Further investigation to identify potential pathway 207 alteration of the auditory tube in the Cavalier King Charles Spaniel would be an interesting addition for 208 further clarification of this disease process.

209

210 The finding of altered thickness of the tympanic bulla in the French Bulldog and English Bulldog with 211 effusion needs to be interpreted with caution. The high percentage of animals with effusion in this study 212 47.1% (80% and 24% respectively for the French Bulldog and English Bulldog), may have influenced 213 measurements. As discussed for the Cavalier King Charles Spaniel, apparent thickening of the tympanic bulla 214 wall has been documented when fluid is present within the middle ear.<sup>11</sup> This results in over estimation of wall 215 thickness. When the thickness of tympanic bulla was compared between animals with and without effusion in 216 the present study by independent sample t-test there was no significant difference though suggesting the 217 results can be trusted. Additionally a recent study also found that the tympanic bulla walls in 3 brachycephalic 218 breeds (Pug, French Bulldog and English Bulldog) were significantly thicker than that of controls.<sup>13</sup> The 219 findings in the latter study related to bulla wall thickness (a mean of 2 mm in the brachycephalic breeds 220 versus a mean of 1 mm in control dogs) are similar to the measurements that we recorded for the French 221 Bulldog (2.5 mm) and Bulldog (1.9 mm). Conflicting their findings though, the bulla wall thickness in Pugs in 222 this study was not significantly different from mesaticephalic controls. These variations may represent bias in 223 patient selection, the variation in presence of middle ear effusion in study groups, or variation in measurement 224 technique. Further investigation into potential breed variations to identify if a true difference is present is 225 therefore warranted.

226

Interestingly, the tympanic bulla volume:weight ratio was significantly different in the Pug, French Bulldog,
CKCS and English Bulldog compared to the Labrador. Although the results in these breeds were not

significant to the JRT the difference approached significance in the comparison between the latter breed and French Bulldogs (p=0.055). We would therefore suggest that although variation in volume exists it does need to be interpreted with caution. Abnormal anatomy, smaller bullae with possibly narrower auditory tubes or altered pathway of the auditory tube could hypothetically predispose brachycephalic breeds to the development of middle ear effusion, especially during episodes of increased upper airway mucosal swelling such as associated with BOAS. Further studies are required to identify if any such relation is present.

235

236 The anatomic position of the middle ear is different in brachycephalic breeds compared to control dogs. Of 237 particular note is the more rostral location with overlap of the tympanic bulla and the temporomandibular joint 238 in the French Bulldog and the Pug (Figure 4 and Table 2). Brachycephaly related skull morphology appears to 239 be a multifactorial and polygenetic trait where some breeds have most changes in the rostral parts of the skull, 240 affecting mainly maxillary growth whereas others apparently have more changes, or concurrent changes, in 241 the caudal bony parts of the skull such as the temporal and occipital bones. Our study seems to indicate that 242 the caudal skull changes are less obvious in the English Bulldog compared to other brachycephalic breeds. 243 This finding highlights the importance of CT as a diagnostic tool for pre-surgical planning when exploration 244 of any area of the skull but the bulla in particular is needed such as for total ear canal ablation and lateral bulla 245 osteotomy. The altered position of the bulla may also offer, as stated above, an additional explanation for 246 middle ear effusion commonly seen in these breeds as the pathway of the auditory tube may be different as a 247 result as well, possibly predisposing these animals to auditory tube dysfunction and leading to the previously high documented rate of subclinical otitis media (12.5 - 41%).<sup>9,14</sup> 248

249

While we were unable to identify a significant association between CT morphometric findings and middle ear effusion in the current study, the authors propose that structural and morphological malformations of the tympanic bullae of brachycephalic dogs may be an important factor in predisposition of the brachycephalic phenotype to auditory tube dysfunction and subsequent fluid accumulation within the middle ear. Otitis media in humans has been linked to brachycephalic type cephalometric measurements and has been shown to decrease in prevalence with maturity due to changes in auditory tube orientation.<sup>10</sup> Fluid can accumulate in the middle ear of humans due to dysfunction of the auditory tube.<sup>15</sup> Experimentally, this process has been induced

in cats and dogs following auditory tube ligation.<sup>16</sup> Otitis media in the latter species has also been associated
with upper respiratory tract infection, palatine defects and with primary ciliary dyskinesia, in which auditory
tube dysfunction was proposed to play an important role.<sup>17-19</sup> Changes in the structural characteristics of the
soft palate, nasopharynx and pharynx in dogs have previously been linked to the incidence of middle ear
effusion.<sup>1,2</sup> We suspect that skeletal variations contribute to an alteration in the path of the auditory tube and
that increased mucosal swelling and mucosal redundancy of the auditory tube lining subsequently predispose
brachycephalic breeds to the development of middle ear effusion.<sup>1,2</sup>

264

There are several limitations inherent to this study. Since a single reviewer (BM) evaluated all studies, it is
possible that some bias may have been present in the interpretation of changes. Repeated measurements to
allow inter-observer and intra-observer assessment would have ideally been performed but this study merely
sought to describe a set of anatomical variations rather than define measurement standards.
The use of mesaticephalic dogs as a comparison groups is another limitation. Finding appropriate weight, sex
and age matched dogs with CT scans of the head with a standardized protocol is unfeasible in a retrospective

study. This study sought to limit confounders with the use of pure-bred dogs of similar weight groups to

animals studied but accept this is a major limitation.

Being retrospective this study also included animals that had scans obtained with slightly different protocols.
Although this may have resulted in slight variation in measurements, we expect that this difference would be
negligible<sup>20</sup>.

276

No histopathological investigation or myringotomy was performed to determine the nature of the soft tissue
attenuation in the middle ear. The authors presumed this to represent middle ear effusion but caution in
overinterpretation is advised. Contrast enhanced CT in addition to myringotomy may have been beneficial in
this scenario.

281

In conclusion, this study sought to identify and describe brachycephalic breed variations in position and shape of the tympanic bulla based on CT morphometric characteristics. Increased bulla wall thickness was detected in the sampled French Bulldogs and English Bulldogs. Significant variations in the tympanic bulla volume

285	were also identified in these breeds. A more rostrally located tympanic bulla with overlap of the tympanic
286	bulla and temporomandibular joint was identified in Pugs and French Bulldogs. There was a significant
287	dorsoventral flattening of the tympanic bulla in the Cavalier King Charles Spaniel. Authors introduced the
288	term "tympanic bulla malformation" and proposed that this be added to the list of brachycephalic phenotypic
289	traits. No significant associations were identified between presence of middle ear effusion and any of the CT
290	morphometric characteristics in this sample of dogs. Future studies are needed to investigate whether there is
291	an association between brachycephalic tympanic bulla malformation and middle ear effusion, and whether
292	presentation for obstructive airway disease predisposes animals to the formation of middle ear effusion.

294	Author Contributions:
295	Category 1
296	(a) Conception and Design
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298	(b) Acquisition of Data
299	Ben Mielke
300	(c) Analysis and Interpretation of Data
301	Ben Mielke, Gert Ter Haar
302	Category 2
303	(a) Drafting the Article
304	Ben Mielke, Gert Ter Haar

- 305 (b) Revising Article for Intellectual Content

Ben Mielke

- 306 Richard Lam, Gert Ter Haar
- 307 Category 3
- 308 (a) Final Approval of the Completed Article
- 309 Ben Mielke, Richard Lam, Gert Ter Haar

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Table 1. Patient parameters; Weight, Sex, Age, and Skull Index. All values are presented asmean ± standard deviation.

Breed	Weight (kg)	Sex Distribution			n	Age	Skull Index
		ME	MN	FE	FS		
Pug (n=25)	8.56 ± 2.16	9	2	7	7	2.83 ±	103.29 ±
						1.67	3.98
French	12.02 ± 1.91	14	8	2	1	2.42 ±	97.29 ± 5.85
Bulldog						2.27	
(n=25)							
Bulldog	23.08 ± 4.98	16	5	2	2	1.90 ±	92.56 ± 5.59
(n=25)						1.99	
CKCS (n=25)	9.21 ± 2.54	5	5	4	11	6.05 ±	79.82 ± 5.52
						2.83	
Labrador	31.98 ± 8.08	2	3	2	6	6.41 ±	60.8 ± 2.28
(n=13)						4.11	
JRT (n=10)	8.94 ± 3.5	2	4	0	4	8.90 ±	57.9 ± 2.37
						3.75	

365 ME: Male Entire, MN: Male Neutered, FE: Female Entire, FS: Female Spayed

Table 2: Breed related Middle Ear Effusion, Internal tympanic bulla Width:Height Ratios,

367 tympanic bulla wall thickness (mm), tympanic bull volume:weight (cm<sup>3</sup>/kg) and tympanic

368 bulla:temporomandibular joint overlap measurements (% skull length)\*.

369

			1		
Breed	Presence of	Tympanic Bulla	Tympanic Bulla	Tympanic	Tympanic
	Effusion	Width:Height Ratio	Wall Thickness	Bulla	bulla:Temporomandibul
		(internal)	(mm)	volume:weight	ar joint overlap
Pug	5/25	$1.26 \pm 0.18^{a}$	0.9 ± 0.3 <sup>d,e</sup>	$0.048 \pm 0.01^{k}$	-1.15 ± 2.21 <sup>f</sup>
_	(20%)				
French	20/25	1.4 ± 0.22 b	2.5 ± 1.3 <sup>d</sup>	0.036±0.01 <sup>k</sup>	-0.5 ± 2.3 <sup>g</sup>
Bulldog	(80%)				
Bulldog	6/25	1.25 ± 0.16 <sup>a</sup>	1.9 ± 0.7 <sup>e</sup>	$0.049 \pm 0.02^{k}$	2.1 ± 3.01 <sup>f,g,i</sup>
_	(24%)				
CKCS	17/25	1.56 ± 0.21 <sup>a</sup>	$1.0 \pm 0.4$ d,e	0.053±0.02 <sup>j</sup>	$0.87 \pm 2.77^{f,h}$
	(68%)				
Labrador	0/13 (0%)	$1.2 \pm 0.07$ <sup>a,b</sup>	1.1 ± 0.3 d,e	0.074±0.01 <sup>j,k</sup>	$4.82 \pm 1.42^{f,g,h,i}$
JRT	0/10 (0%)	1.25 ± 0.16 <sup>a</sup>	$0.8 \pm 0.1$ <sup>d,e</sup>	0.065±0.01	3.5 ± 1.14 <sup>f,g,h</sup>

370 \* Values are presented as mean ± standard deviation. Superscript represents significant

differences between breeds (p<0.05).

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- 376 FIGURE LEGENDS:
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- 378 Figure 1: Transverse Computed tomographic scan images for measurement of tympanic bulla
- height and width obtained at level of tympanic window. Solid line H: internal height of
- tympanic bulla (HTB1), W: internal width of tympanic bulla (WTB1). Dashed line; external
- 381 width of tympanic bulla (WTB2)



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Figure 2: Dorsal plane multiplanar reconstruction image demonstrating the relative position of
the tympanic bulla and relevant anatomy. Asterisk (\*) denotes the prosthion, Arrow heads:

387 medial temporo-mandibular joint, Arrows: Rostral internal and caudal internal tympanic bulla



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- 390 Figure 3: Comparison of shape of tympanic bulla between the Labrador (left) and CKCS (right).
- Note the flatter appearance of the CKCS with mean  $WHR_{int} = 1.56 \pm 0.21$  compared to Labrador
- 392 WHR<sub>int</sub> =  $1.2 \pm 0.07$



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- 395 Figure 4: 3D reconstruction images of the skull viewed from a ventral orientation. Solid black
- 396 lines mark the position of the temporomandibular joint. Dotted black lines show the
- 397 craniocaudal extent of the tympanic bulla. A) French Bulldog, B) Pug and C) English Bulldog D)
- 398 Cavalier King Charles Spaniel E) Jack Russell Terrier F) Labrador

