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1	TITLE:	ULTRASONOGRAPHIC	IDENTIFICATION	OF	THE	DORSAL
2	ATLANT(DAXIAL LIGAMENT IN DO	GS			

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22 Ultrasonographic identification of the dorsal atlantoaxial ligament in dogs

23 ABSTRACT

24 OBJECTIVE: The purpose of this study was to evaluate if ultrasonography is a feasible tool

- to identify the dorsal atlantoaxial ligament in dogs.
- 26 STUDY DESIGN: Canine cadaveric study.
- 27 SAMPLE POPULATION: Canine cervical spines (n=35)

METHODS: Thirty-five canine cadavers with an estimated body weight of 6-35kg were retrieved. Three cervical spines were dissected to demonstrate the dorsal aspect of the atlantoaxial joint and assess the length and thickness of the dorsal atlantoaxial ligament. Thirty cadavers were used for the ultrasonographic evaluation of the dorsal atlantoaxial ligament and a subjective score (0-3) was assigned to each dog depending on the visibility of the dorsal atlantoaxial ligament in both the transverse and the sagittal planes.

RESULTS: The dorsal atlantoaxial ligament was detectable on ultrasound in all cadavers: 27/30 and 28/30 were graded as moderately visible (grade 2) or clearly visible (grade 3) in the sagittal and transverse view respectively. Only 1/30 cadaver specimen of a large breed dog was graded as 1 (indistinct) in both the sagittal and transverse planes. None of the cadavers were graded as 0 (not visible) in any view.

CONCLUSIONS: Ultrasonographic identification of the dorsal atlantoaxial ligament is a
feasible technique in normal canine cadavers. Future studies on patients clinically affected
from atlantoaxial instability/subluxation need to be done to evaluate the role of this
diagnostic tool in a safer diagnosis of this condition.

43 CLINICAL RELEVANCE: Identification of the dorsal atlantoaxial ligament through
44 ultrasonography could potentially diagnose patients with atlantoaxial instability/subluxation
45 using a non-invasive and safe diagnostic imaging technique.

47 INTRODUCTION

Atlantoaxial instability is a common condition of the cervical spine which can result in subluxation of the axis in relation to the atlas and subsequent spinal cord compression^{1,2,3,} Atlantoaxial instability/subluxation is a potentially life-threatening condition⁴. Patients with this condition develop clinical signs at a young age, with 52-70% of patients being less than 1 year old⁵. Clinical signs vary from neck pain and mild ataxia in 24.9% of cases to tetraplegia in 6.5% of cases¹.

Atlantoaxial subluxation has been reported in 38 breeds of dogs, being most common 54 in Yorkshire terriers (28%), Toy Poodles (18%) and Chihuahuas (15% of cases)⁶. Congenital 55 atlantoaxial subluxation is most commonly seen in small and toy breed dogs, but can also be 56 seen in medium and large breed dogs^{7,8,9,10,11,12}. These animals with atlantoaxial congenital 57 abnormalities will have atlantoaxial subluxation either spontaneously or with minimal 58 trauma¹³. Traumatic atlantoaxial subluxation can occur in any breed of dog² with one of the 59 possible causes being traumatic rupture of the atlantoaxial ligaments during a forceful 60 overflexion of the neck 2 . 61

Diagnosis of atlantoaxial subluxation is based on clinical evaluation of the patient, 62 and is confirmed by various imaging techniques (plain radiographs, CT or MRI). One of the 63 simplest methods is identifying an increased space between the dorsal lamina of the atlas and 64 the spinous process of the axis^{2,5} on plain radiographs, which indirectly demonstrates the 65 dorsal atlantoaxial ligament. Flexed cervical radiographs can be done in those cases where 66 67 the increase space cannot be detected on neutral position⁵, but this should be done with extreme care as it could lead to further compression of the spinal $cord^2$ and deterioration in 68 the clinical status of the patient. Although myelography can been used to diagnose 69 atlantoaxial subluxation, this technique is no longer recommended due to the risks associated 70 to myelograms and the accessibility to other diagnostic techniques¹. Advanced diagnostic 71

72 techniques such as MRI and CT can provide additional information regarding spinal cord injury¹⁴ and osseous abnormalities¹⁵ in the atlantoaxial joint, being also useful for surgical 73 planning¹. However these techniques are expensive and have disadvantages such as requiring 74 75 a long anaesthetic period (MRI) or exposing the patient to an increased radiation dose (CT). In contrast to these techniques, ultrasonography is a fast, safe and relatively inexpensive 76 technique. Ultrasonography has been used to assess the craniocervical junction in animals 77 with Chiari-like malformation and syngomyelia^{5, 16, 17} and intracranial arachnoid cysts¹⁸. 78 Ultrasonography has also been used to evaluate the musculoskeletal anatomy of the dorsal 79 cervical spine¹⁹. However, information regarding the use of ultrasonography to assess 80 atlantoaxial ligaments is lacking. 81

The dorsal atlantoaxial ligament courses from the dorsal lamina of the atlas to the most cranioventral aspect of the spinous process of the axis, and this thick dorsal atlantoaxial ligament contributes to the stability of the atlantoaxial joint²⁰. As this is the exact location where the increase in intervertebral space is seen radiographically in those patients with atlantoaxial subluxation, it has been suggested that the increase in space is allowed by the stretching or the rupture of the dorsal atlantoaxial ligament^{2, 20, 21}.

The objective of this study was to evaluate if ultrasonography was a feasible tool to identify and evaluate the integrity of the dorsal atlantoaxial ligament. Based on the relative small size of the ligament and its anatomical location we hypothesised that the dorsal atlantoaxial ligament would not be detectable on ultrasonography.

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97 MATERIALS AND METHODS

Ethical approval for the study was obtained from the authors' institution. Thirty five adult 98 canine cadavers of dogs euthanized for reasons unrelated to this study were used. The 99 100 cadavers had been initially frozen for several days and were allowed to thaw at room temperature prior to our study. Initially 3 cadavers were dissected to demonstrate the dorsal 101 aspect of the cervical spine and assess the length and thickness of the dorsal atlantoaxial 102 ligament (Figure 1), and to subjectively determine if it would be of sufficient size and in an 103 adequate location to be detected by ultrasonography. A further two cadavers were used to 104 105 correlate the ultrasonographic findings with the anatomy of each specimen. Ultrasound guided injection of ink into the suspected dorsal atlantoaxial ligament was followed by 106 dissection of these 2 cadavers. Thirty adult canine cadavers were then collected and used for 107 108 the ultrasonographic evaluation of the dorsal atlantoaxial ligament. The cadavers were positioned in lateral recumbency and the dorsal cervical area was clipped at the level of the 109 atlantoaxial joint. The skin was cleaned with spirit and gel was applied. 110

Ultrasonography was carried out using an 8 MHz microconvex transducer, with the 111 cadavers positioned in right lateral recumbency with a neutral or slightly flexed cervical 112 position. The transducer was placed on the dorsal aspect of the neck caudal to the occipital 113 crest, and the cranial aspect of the spinous process of C2 was identified. The transducer was 114 placed in a transverse plane to identify the spinous process of C2 and then moved cranially to 115 116 identify the dorsal atlantoaxial ligament as a thin hyperechoic structure with linear striations running in a cranioventral direction from the cranial tip of the spinous process of C2 to the 117 dorsocaudal aspect of the dorsal lamina of C1. Once the ligament had been identified it was 118 119 also scanned in a straight sagittal plane (Figures 2 and 3). All the ultrasonographic studies were performed by a board-certified specialist in diagnostic imaging. A subjective score (0-3) 120 was assigned to each dog depending on the detectability of the dorsal atlantoaxial ligament 121

122	and its differentiation from surrounding structures in both the transverse and the sagittal
123	plane. This grading score was similar to the classification used in previous cadaveric
124	studies ²² . Those cadavers in which the dorsal atlantoaxial ligament was clearly identified
125	based on the expected location, fibre orientation and fibre echogenicity were assigned a grade
126	3, if it was moderately visible they were assigned a grade 2, if the margins of the ligament
127	were indistinct but an hypoechogenic band could be identified in the expected location of the
128	ligament they were assigned a grade 1 and they were assigned a grade 0 when the fibres of
129	the dorsal atlantoaxial ligament were not discernible at ultrasonographic evaluation.
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147 **RESULTS**

Anatomical dissection of the two cadavers in which coloured ink was injected (ultrasound guided) in the ligament showed the presence of the injected ink in close association with the dorsal atlantoaxial ligament (with some leakage from the ligament suspected), confirming the accuracy of the ultrasonographic findings.

All the cadavers were mature adult dogs, but the exact age was unknown. The majority of dogs were crossbreed dogs, while there were 3 German shepherd, 1 Labrador, 1 Rottweiler, 1 Rough Collie and 1 Husky. The estimated weight of the dogs ranged between 6 and 35kg. Dogs were classified as small (estimated body weight <10kg), medium (estimated body weight 10-25 kg) or large (body weight>25kg). In total 19 cadavers were large breed dogs, 10 cadavers were medium size dogs and 1 cadaver was a small breed dog. Table 1 summarizes the results.

Of the 19 large size cadavers 10 were graded as 3 either in the sagittal, in the transverse or in both planes. Eight of the other large breed cadavers were graded as 2 either in the sagittal, in the transverse or in both planes, while 1 cadaver was graded as 1 in both the sagittal and the transverse plane. None of the large size cadavers were graded as 0 in any of the planes.

Of the 10 medium size cadavers 5 were graded as 3 either in the sagittal, in the transverse or in both planes and 5 were graded as 2 in either in the sagittal, in the transverse or in both planes. None of the medium size cadavers were graded as 0 or 1 in any of the planes.

168 Finally, the only small size cadaver was graded as 3 in both the sagittal and the 169 transverse plane.

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172 **DISCUSSION**

173 Results from our study show that ultrasonography is a useful diagnostic tool to identify the
174 dorsal atlantoaxial ligament in normal canine cadavers, and the ligament was detectable in all
175 cadavers.

In our study none of the cadavers were graded as 0 (not visible) in any view and 27/30 176 and 28/30 were graded as moderately visible (grade 2) or clearly visible (grade 3) in the 177 178 sagittal and transverse view respectively. Only 1 cadaver specimen of a large breed dog (Husky) was graded as 1 (indistinct) in both the sagittal and transverse plane, while in the 179 180 other 3 dogs (all large size) in which the identification of the ligament was graded as 1 in one view it was graded as 2 in the other view. Therefore, 29/30 specimens were graded at least 2 181 in one ultrasonographic view and 16/30 had at least one view graded as 3, with 7/30 being 182 183 graded 3 in both views, 10/30 being graded 2 in both sagittal and transverse views and 9/30 being graded 2 in one view and 3 in the other view. Interestingly, none of the medium size 184 dogs was graded as 1 in any of the 2 ultrasonographic views, and the only small breed 185 cadaver was graded as 3 in both views. These results suggest that visualization of the 186 ligament might be clearer in smaller breeds. The reason for this finding is not clear from this 187 study, but the authors hypothesise that this could be due to a proportionally smaller muscle 188 mass surrounding the ligament in smaller breeds compared to large breeds, particularly those 189 larger dogs with a bull terrier type conformation. Many of the larger-breed cadavers in this 190 191 study did have a fairly broad skull conformation, so there may be a degree of bias due to this. An alternative hypothesis would be a proportionally larger dorsal atlantoaxial ligament in 192 smaller breeds allowing proportionally clearer visualisation. As atlantoaxial subluxation is a 193 194 condition commonly diagnosed in small breed dogs, in which there is typically a congenital origin, further studies will need to determine the feasibility of this technique in small breed 195 dogs. Most of the breeds in this study were medium or large breed dogs, and therefore this 196

study shows that ultrasonography could be a useful diagnostic tool for these breed of dogs, in
which the congenital form of atlantoaxial instability/subluxation is less common and are more
likely to have the traumatic presentation of atlantoaxial subluxation.

200 In order to ultrasonographically identify and assess the echogenicity of a ligament it is necessary to maintain the ultrasound beam perpendicular to the ligament^{23, 24}. In our study 201 the acoustic window was considered small due to the large size of the spinous process of the 202 203 axis, and therefore it is possible that the amount of ligament identified did not represent the full extent of the dorsal atlantoaxial ligament. On the other hand, the ultrasonographic 204 205 evaluation was done with the neck in neutral position or slightly flexed, and it is likely that identification of the dorsal atlantoaxial ligament would have been better if the neck was in a 206 flexed position, as there would be less overlapping of the spinous process of the axis over the 207 208 atlas. However, the authors decided to do the ultrasonographic evaluation of the dorsal 209 atlantoaxial ligament in neutral or slightly flexed positions as these would be the more clinically relevant positions, avoiding the risk of flexing the neck in clinically affected 210 patients. An 8-10Mhz linear array transducer was briefly used to try to identify the ligament 211 at the beginning of the study, but the clarity of the resulting images of the ligament was poor, 212 with the microconvex array performing noticeably better, and hence this type of transducer 213 was used. The authors suspect that this is due to the conformation of the spinous process of 214 the axis resulting in the ligament being partially masked by the acoustic shadow from the 215 216 process, and the footprint of the linear array preventing easy angulation to improve image quality due to the surrounding musculature. The fan-shaped image generated by the 217 microconvex array, coupled with the smaller footprint, allowed a clear image of the ligament 218 219 to be obtained. As the transducer was positioned in a straight sagittal position, further studies will be required to assess if positioning the transducer in a parasagittal position to obtain 220 oblique views of the dorsal atlantoaxial ligament improves its assessment in dogs. 221

Five specimens were used to assess the feasibility of this technique. Three specimens 222 were used for anatomical dissection to subjectively assess the dimensions and exact location 223 of the dorsal atlantoaxial ligament. Two further specimens had coloured ink injected into the 224 225 dorsal atlantoaxial ligament under ultrasound guidance followed by anatomical dissection. Although the total number of cadavers used for these assessments was relatively small, the 226 authors considered that the findings in the cadavers were very consistent regarding the 227 subjective dimensions and position of the dorsal atlantoaxial ligament. The location of the 228 coloured ink in the ligament was very accurate in the 2 cadavers used for this purpose, 229 230 confirming that the ultrasonographic identification of the dorsal atlantoaxial ligament was correct. Even though only 2 cadavers were used for this purpose, the authors considered this 231 number sufficient due the accuracy found in the ultrasound guided ink injection when 232 233 dissecting both cadavers and the characteristic ultrasonographic images found at the expected anatomical location of the dorsal atlantoaxial ligament. 234

The authors acknowledge several limitations to this study. First, we did not perform 235 anatomic dissection of the dorsal atlantoaxial ligament of all the cadaveric specimens because 236 the purpose of the study was only to determine whether ultrasound imaging can depict the 237 dorsal atlantoaxial ligament. We therefore acknowledge that although the breeds of the 238 cadavers were not breeds predisposed to atlantoaxial instability/subluxation it is possible that 239 some of the cadavers did actually have abnormalities in the dorsal atlantoaxial ligament. 240 241 Secondly, the study was performed on previously frozen cadavers and to the authors knowledge there are no studies assessing the influence of the process of freezing/thawing 242 cadavers on the ultrasonographic image of a ligament. Therefore it is possible that this could 243 244 have affected the ability of the operator to identify the dorsal atlantoaxial ligament. Finally, no attempts were made to perform measurements in the ultrasonographic images and 245 correlate them to anatomic dissections as the purpose of the study was to assess if 246

identification of the dorsal atlantoaxial ligament was possible. The authors performed the study trying to mimic the clinical scenario and only allowing a mild flexion of the neck of the cadavers. In order to measure the full extent of the ligament the dorsal atlantoaxial ligament would have need to be taut by fully flexing the head, which would invalidate the potential benefits of this technique compared with traditional plain radiographs in the diagnosis of atlantoaxial subluxation/instability.

In conclusion, this study demonstrates that ultrasonographic identification of the 253 dorsal atlantoaxial ligament is a feasible technique in normal canine cadavers weighing more 254 255 than 6 kg. Therefore, identification of the dorsal atlantoaxial ligament through ultrasonography could potentially diagnose patients with atlantoaxial instability/subluxation 256 using a non-invasive and safe diagnostic imaging technique. This technique has the 257 258 advantage over conventional radiographs that it can be performed with the neck at a neutral or slightly flexed position. Further studies are needed to assess the feasibility to identify the 259 dorsal atlantoaxial ligament in live and numerous small breed dogs and correlate the findings 260 261 with patients clinically affected by atlantoaxial instability/subluxation.

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- 273 None

297 DISCLOSURE

298 The authors declare no conflicts of interest related to this paper.

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397 FIGURE AND TABLE LEGENDS

399	Figure 1: Anatomic dissection of the dorsal aspect of the atlantoaxial joint showing the
400	anatomic location of the dorsal atlantoaxial ligament (star)
401	Figure 2: Ultrasonographic sagittal image of the dorsal aspect of the atlantoaxial joint
402	showing the cranial aspect of the spinous process of the axis (x), the dorsal atlantoaxial
403	ligament (arrowhead) and the dorsal arch of the atlas (star)
404	Figure 3: Ultrasonographic transverse image of the dorsal aspect of the atlantoaxial joint
405	showing the spinous process of the axis (x), the dorsal atlantoaxial ligament (arrowhead) and
406	the dorsal arch of the atlas (star)
407	Table 1: Assigned grades in the sagittal and transverse ultrasonographic views according to
408	the size of the cadavers.
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422 TABLE 1

				Assigned grade						
	Clearly	visible (3)	Moderat	ely visible (2)	Indistinct (1)		Not visible (0)			
	Sagittal	Transverse	Sagittal	Transverse	Sagittal	Transverse	Sagittal	Transverse		
Large breed (n=19)	8	6	8	11	3	2	0	0		
Medium breed (n=10)	4	3	6	7	0	0	0	0		
Small breed (n=1)	1	1	0	0	0	0	0	0		





