Influence of ventral fixation techniques on atlantoaxial joint fusion in canine models with dens partial resection

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ABSTRACT. We evaluated the completeness of bony fusion of the atlantoaxial joint (AAJ) through polymethylmethacrylate fixation (PMF) and atlantoaxial plate fixation (APF) using six canine models with dens partial resection. In both groups, the hydroxyapatite content at the AAJ was measured up to 7 months postoperatively using quantitative computed tomography. Histological assessment revealed fibrous fusion in the PMF group. Meanwhile, in the APF group, only one dog achieved fibrous fusion, whereas the remaining three showed bony fusion. To our knowledge, this study was the first to evaluate AAJ fusion histologically after PMF and APF. The present study demonstrates that PMF and APF may stabilize the AAJ without clinical complications. Therefore, PMF and APF are clinically useful fixation methods for atlantoaxial instability.

KEYWORDS: atlantoaxial instability, atlantoaxial plate fixation, bony fusion, fibrous fusion, polymethylmethacrylate fixation

Atlantoaxial instability (AAI), which commonly affects young toy breed dogs, results in various degrees of cervical spinal cord injury associated with dynamic instability of the atlantoaxial joint (AAJ) [2, 4, 6, 12, 23, 27–30]. We previously reported that atlantoaxial plate fixation (APF) using an AAJ fixation plate (Platon Japan Co., Ltd., Tokyo, Japan) resulted in inferior flexural strength and superior torsional strength compared to those of multiple metallic implants and polymethylmethacrylate (PMMA) fixation (PMF) [26]. The maximum load for the APF varied slightly because the screw position and angle were constant. Other studies have reported that PMF is an effective ventral fixation technique adapted for AAI management in dogs with various vertebral builds and shapes [1, 8]. The disadvantages of using PMMA include polymerization heat, infection, mechanical pressure on the esophagus and trachea, and implant breakage [3, 11, 16]. Since the AAJ has torsional mobility centered on the dens of the axis, ensuring resistance during flexion and torsion is important until the AAJ fuses [26]. Considering the drawbacks of PMMA and that the resistance to torsion was the greatest at the AAJ in a mechanical strength test, APF has been considered an alternative fixation method to PMF [26]. Ventral stabilization of the AAJ is performed for AAI management by using PMF and APF [1, 5, 8, 10, 14, 17–19, 25, 29, 30]. However, common methods to assess the outcomes of ventral stabilization of the AAJ rely on postoperative neurological evaluation or assessment of implants using radiography. The final goal of ventral stabilization of the AAJ is the bony fusion of the AAJ [20–22]. However, studies seldom evaluate the occurrence of bony fusion of the AAJ. Sorjonen *et al*. suggested the need for histological evaluation of AAJ fusion in the presence or absence of transarticular fixation in dogs with induced AAI [24]. However, to the best of our knowledge, histological evaluation of AAJ fusion after PMF and APF, both widely used, has not been reported. This study aimed to evaluate the completeness of bony fusion of the AAJ through PMF and APF using canine models with partial dens resection.

This study included six healthy beagles with no abnormalities in general physical, blood, orthopedic, neurologic, or radiologic examinations. We performed anesthesia, surgical management, and euthanasia of the dogs according to the guidelines for the care and use of laboratory animals at our university (Approval No. S26S-15, 27S-2).

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We anesthetized the dogs and ventrally approached the AAJ using the techniques reported by Schulz *et al*. [19] and Shores *et al*. [23]. For analgesic management, we administered butorphanol tartrate (Meiji Seika Pharma Co., Ltd., Tokyo, Japan; 0.1 mg/kg) intramuscularly and buprenorphine (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan; 0.02 mg/kg) intravenously. A 5-mm bone resection was performed on the basal portion of the dens using an electric microdrill (Electric Pen Drive, Johnson & Johnson DePuy Synthes, Tokyo, Japan) by partial resection of the dens, and the cranial portion of the dens was completely separated from the body of the axis. After partial dens resection, three dogs underwent PMF, whereas the remaining dogs underwent APF using a modified fixation method described previously [26]. The improved PMF method used two φ2.4-mm cortex screws (Mizuho Co.; length: 16 mm) for the axis, but the same implants were made of stainless steel (SUS316L), as reported previously [26]. We installed these fixation screws such that only half of the length, not beyond the thickness of the longus coli muscle, was prominent from the surface of the bone. The APF method, which we developed previously [26], was performed using an AAJ fixation plate (Platon Japan Co., Ltd.) made of pure titanium (ASTM F67 grade 2), which we further improved to be able to use locking screws in most caudal screw holes of the plate. Two φ2.0-mm locking screws (Platon Japan Co., Ltd.; length: 18 mm) were inserted into these screw holes using the same implants made of titanium alloy (ASTM F136), as reported previously [26]. The plate was designed and served as a prototype of the atlantoaxial fixation plate to fit a beagle dog weighing 10–15 kg, based on the DICOM CT data of beagle dogs, for safe and secure ventral fixation of the AAJ in dogs (Fig. 1). In particular, we designed the screw hole on the cranial side of the plate to incline 5° and the most caudal side to incline 15° outward to avoid the spinal canal. In both groups, cancellous bone (approximately 1,000 mm^3) was measured using a syringe, harvested from the major tubercle of the humerus, and grafted into the gap of the AAJ with the articular cartilage removed. In the PMF group, fixation was achieved using PMMA (Stryker Co., Kalamazoo, MI, USA; approximately 15 cm^3) and measured using a feeding syringe (TOP Co., Tokyo, Japan) with a large nozzle diameter.

The postoperative assessment was performed under general anesthesia using radiography and computed tomography (CT) immediately; 2 weeks; and 1, 2, 3, 4, 5, 6, and 7 months postoperatively. We used radiography and CT to evaluate the implant integrity and CT to evaluate the hydroxyapatite (HA) content of the AAJ. After 7 months of observation, we euthanized the dogs by intravenous administration of pentobarbital (Kyoritsu Seiyaku Co., Tokyo, Japan; 50 mg/kg).

CT was performed using an 80/160-slice CT scanner (Aquilion PRIME-TSX-303A, Toshiba Medical Systems Co., Otawara, Japan) set at 120 kV and 150 mA, with a 0.5-sec rotation time and 0.5-mm slice thickness. The data were analyzed using commercial image-processing software (Osirix, Pixmeo, Geneva, Switzerland). DICOM CT data were used to perform a three-dimensional multiplanar reconstruction at window widths and levels of 2,500 and 500 Hounsfield units, respectively [13]. After setting the region of interest between the caudal part of the ventral atlas and cranial side of the vertebral body of the axis in the median sagittal image, the average postoperative CT value was measured (Fig. 2A and 2C). A standard bone mineral phantom (B-MAS 200, Kyoto Kagaku Co., Ltd., Kyoto, Japan) was used to evaluate the HA content in the AAJ gap using quantitative CT (QCT) [7].

After euthanizing the dogs, we obtained specimens by amputating the atlanto-occipital joint and the joint between the axis and the third cervical vertebrae. We cleared the atlas–axis portion with implants of surrounding soft tissue as much as possible and then removed the implants. Next, we fixed the samples in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA). Subsequently, the obtained atlas–axis portion was cut mid-sagittally through the remaining cranial part of the dens and fovea dentis of the AAJ and embedded in paraffin wax, according to conventional methods. We then sectioned the samples at 5 μm thickness and stained them with hematoxylin and eosin (HE) for histopathological observation. To assess the distribution of collagen fibers and cartilage matrix, we stained the sections with Masson's trichrome (MT) or safranin-O/fast green (SF). Tartrate-resistant acid phosphatase (TRAP) staining was performed to examine the osteoclast content, according to the method described by Wijngaert *et al*. [31]. Immunohistochemistry for runt-related transcription factor 2 (RUNX 2), an osteoblast marker, was performed to evaluate the number of osteoblasts using an anti-RUNX 2 goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) [9]. We evaluated each sample using an upright microscope (BX51; Olympus Co., Tokyo, Japan) and captured digital images. From
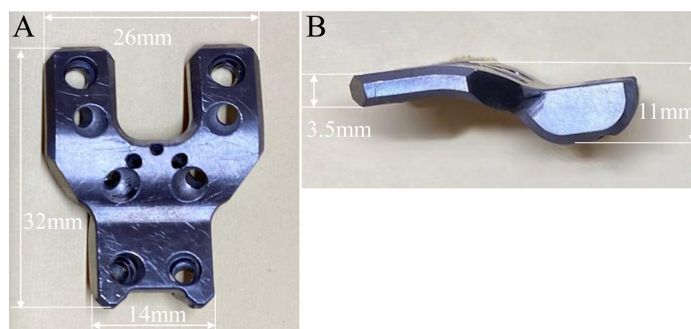

Fig. 1. Atlantoaxial fixation plate (**A**: ventral aspect. **B**: lateral aspect). The plate was designed and served as a prototype of the atlantoaxial fixation plate to fit a beagle dog weighing 10–15 kg, based on the DICOM computed tomography data of beagle dogs, for safe and secure ventral fixation of the atlantoaxial joint.
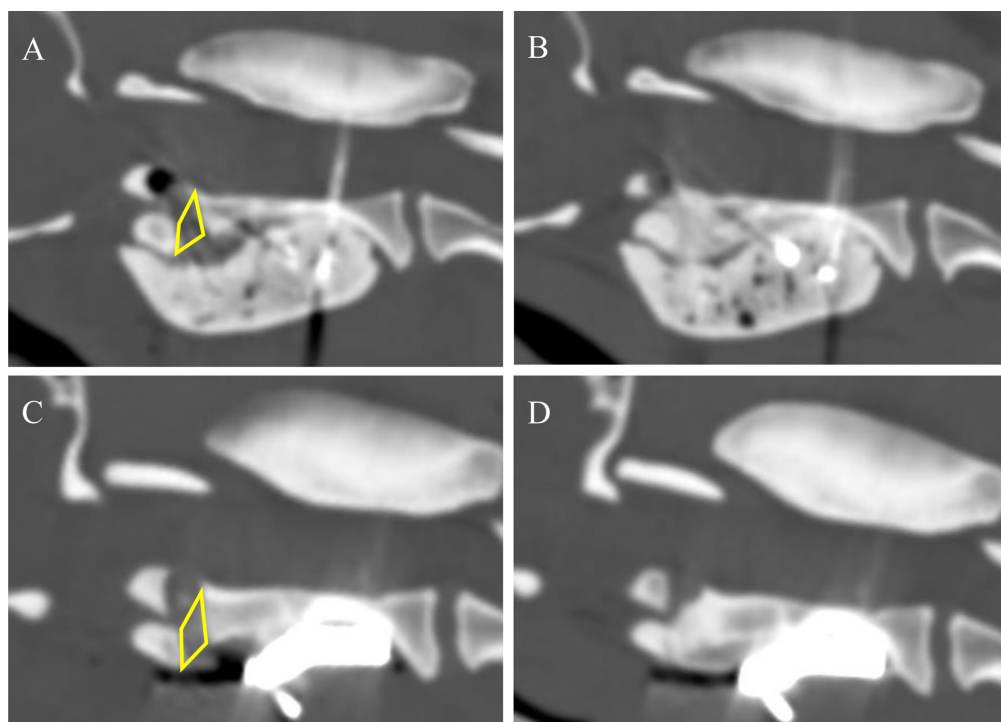
Fig. 2. Postoperative computed tomography sagittal images in the two groups: polymethylmethacrylate fixation (PMF) and atlantoaxial plate fixation (APF). **A**) Dog No. 1 (PMF) immediately after surgery, **B**) Dog No. 1 (PMF) 7 months postoperatively, **C**) Dog No. 6 (APF) immediately after surgery, **D**) Dog No. 6 (APF) 7 months postoperatively. The mean computed tomography value of the gap of the atlantoaxial joint (caudal part of the ventral atlas and cranial side of the vertebral body of the axis between the separation of the dens) was measured at the region of interest (yellow frame) in sagittal images that underwent three-dimensional multiplanar reconstruction in the bone window setting (window width, 2,500 Hounsfield units; window level, 500 Hounsfield units).

the captured digital images, the samples were qualitatively assessed using HE, MT, and SF staining. Areas of fibrous tissue and cartilage matrix in the AAJ gap were measured using an image analysis software (cellSens, Olympus Co.) for microscopic quantitative assessment. However, it was difficult to distinguish between the existing and neonatal bones; therefore, we measured the area of the bone tissue in the entire sample. Furthermore, we observed the TRAP-stained samples thoroughly and counted the number of TRAP-positive osteoclasts. Tissue samples were stained with RUNX2-IH, 10 visual fields were observed at ×200 magnification, and the RUNX2-positive cells were counted. Bony fusion was defined as AAJ infiltration of chondrocytes or osteocytes, whereas fibrous fusion was AAJ infiltration of collagen fibers. Tissue specimens were evaluated by a blinded histopathologist.

All experimental animals were intact male beagles. The mean age (in months) of the dogs in the APF group was 11.7 ± 0.6 months (median, 12.0 months; range, 11–12 months). All dogs in the PMF group were 10 months of age. The mean body weight of the dogs was 12.3 ± 0.9 kg (median, 12.2 kg; range, 11.4–13.2 kg) in the APF group and 10.1 ± 0.8 kg (median, 10.2 kg; range, 9.2–10.8 kg) in the PMF group.

There were no implant failures observed in the APF group; however, we identified some implant breakage in two dogs in the PMF group during the 2-month postoperative assessment. No additional implant breakage was observed from 2 months postoperatively until study completion. No particular clinical signs were associated with implant breakage.

We assessed the mean HA content of the AAJ using QCT. In both groups, mean HA content increased over time throughout the observation period (Fig. 3).

Qualitative assessment of tissue samples obtained from the AAJ using HE, MT, and SF staining (Fig. 4) revealed that collagen fibers, which stained deep blue with MT, infiltrated tissue samples from one dog in the APF group. In another dog, chondrocytes that stained deep red with SF accounted for most of the content. Tissue samples from the remaining dogs in the APF group were infiltrated by chondrocytes and osteocytes with a morphology similar to that of the bone matrix of the atlas and axis visualized by HE staining. Within the PMF group, all three dogs primarily had collagen fibers. Tissue samples from one dog in the PMF group were mildly infiltrated by chondrocytes within collagen fibers. Table 1 presents the results of the quantitative evaluation using HE, MT, and SF staining. The mean number of TRAP-positive osteoclasts per 100 mm^2 in the PMF group calculated from the total area of each sample (PMF group, 127.7 ± 30.6 mm^2; APF group, 132.7 ± 33.8 mm^2) was 19.2 ± 14.2, while that in the APF group was 16.8 ± 12.9. When we observed the tissue samples in 10 visual fields at ×200 magnification, the mean number of cells with RUNX2-IH expression stain was 29.1 ± 7.0 in the APF group and 45.7 ± 54.6 in the PMF group.
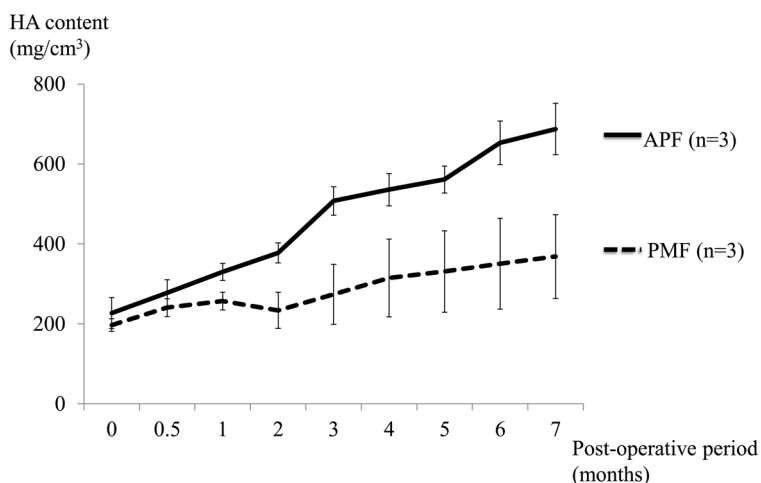
Fig. 3. Comparison of the mean hydroxyapatite (HA) content of the atlantoaxial joint gaps between the two groups: polymethylmethacrylate fixation (PMF) and atlantoaxial plate fixation (APF). In the APF and PMF groups, the mean HA content increased over time throughout the observation period and tended to be higher in the APF group than in the PMF group.
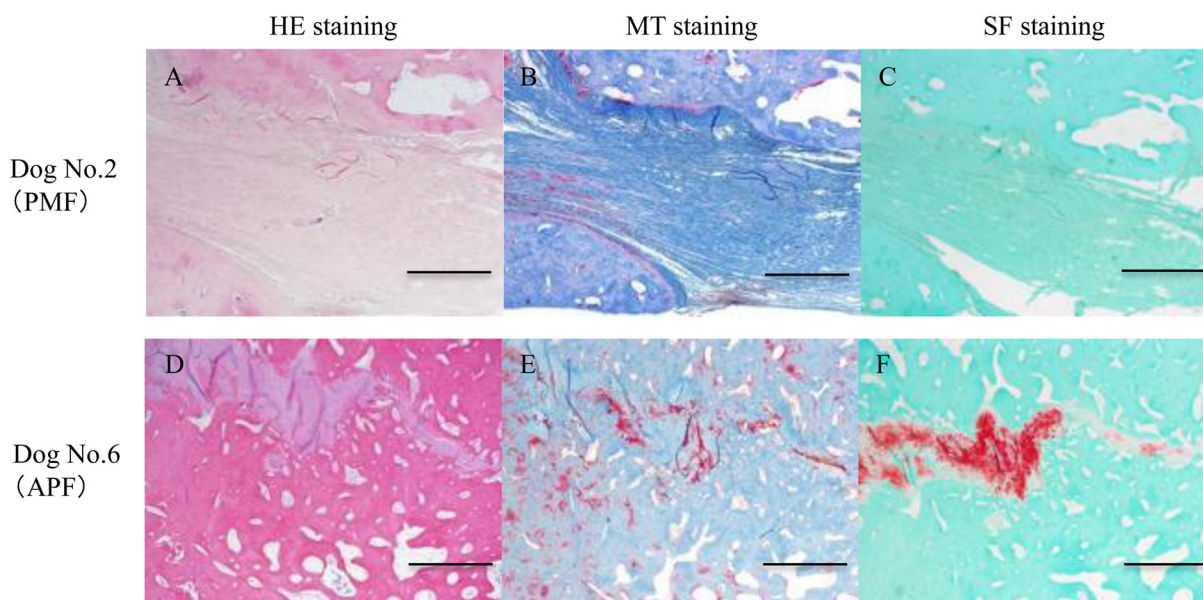

Fig. 4. Histological evaluation of the ventral midline gap of the atlantoaxial joint using hematoxylin & eosin (HE), Masson's trichrome (MT), and safranin-O/fast green (SF) staining in the two groups: polymethylmethacrylate fixation (Dog No. 2) and atlantoaxial plate fixation (Dog No. 6). The first row (**A** and **D**) from the left shows the HE staining performed to evaluate the overall tissue structure. The second row (**B** and **E**) from the left shows the MT staining performed to evaluate collagen distribution, which stained deep blue. The third row (**C** and **F**) from the left shows the SF staining performed to evaluate the cartilage matrix, which stained deep red. The scale bar indicates 100 μm.

In this study, we harvested the AAJ 7 months postoperatively and assessed the tissue histologically. The apical odontoid and alar ligaments are attached to the cranial aspect of the dens; therefore, rupture of these ligaments dorsally displaces the dens and compresses the spinal cord. The shear load is a force associated with supporting the weight of the head, and the alar ligament resists this load at the AAJ [15]. Dens dysplasia is common in dogs with AAI [2, 21, 28]. In these cases, rotational braking of the AAJ by the apical odontoid and alar ligaments and hyperflexion braking of the AAJ by the transverse ligament of the atlas do not function properly.

Two months postoperatively, we confirmed partial implant failures in two dogs in the PMF group, none of whom were free of postoperative neurological abnormalities or experienced further implant-associated complications. No implant failure occurred in the APF group. This suggests that while the AAJ fixation plate used for APF had adequate fixation strength *in vivo*, the fixation strength of the PMF was inadequate for repetitive loads. In a previous study [26], PMF was superior to APF in terms of initial fixation strength; however, no repeated load test was performed. Furthermore, PMF has the risk of heat generation during the

Table 1. Results of quantitative evaluation by hematoxylin-eosin staining, masson trichrome staining and safranin-O/fast green staining

Group	Dog No.	Hematoxylin-eosin staining			Masson trichrome staining			Safranin-O/fast green staining		
		Area of the entire specimen (mm^2)	Area of bone tissue of the entire specimen (mm^2)	Area rate (%)	Area of the entire specimen (mm^2)	Area of fibrous tissue within the gap of the AAJ (mm^2)	Area rate (%)	Area of the entire specimen (mm^2)	Area of the cartilage matrix deposition site within the gap of the AAJ (mm^2)	Area rate (%)
PMF	1	127.3	89.7	70.5	116.4	23.4	20.1	128.4	0	0
	2	94.5	50.8	53.7	91.9	21.1	22.9	89.3	0	0
	3	163.5	104.2	63.8	166.5	22.9	13.8	163.6	3.7	2.3
APF	4	107.1	77.0	71.9	104.1	25.9	24.9	106.3	0	0
	5	170.3	96.2	56.5	160.5	33.4	20.8	168.4	13.6	8.1
	6	121.7	98.3	80.8	119.6	11.1	9.3	120.3	1.7	1.4

PMF: polymethylmethacrylate (PMMA) fixation, APF: atlantoaxial plate fixation.

polymerization of PMMA, thus affecting bony fusion. Heat polymerization delays bony fusion, which increases the early load on anchor implants and causes early implant failure.

QCT findings over 7 months showed that both groups experienced an increase in HA content over time. The histological assessment showed AAJ infiltration of collagen fibers in the PMF group, suggesting fibrous fusion. In contrast, in the APF group, fibrous fusion and infiltration by collagen fibers were observed in only one dog. Samples from the remaining two dogs in the APF group showed infiltration by chondrocytes or osteocytes, suggesting bone fusion. A considerable number of cells that stained positive for RUNX2-IH may have differentiated into osteoblasts, which promoted bony fusion [9]. A more active tissue response may be identified if histological assessments are conducted earlier than 7 months postoperatively, as in this study. Furthermore, histological agglutination may continue if a long-term histological evaluation is performed. In addition, two dogs with partial implant failures in the PMF group tended to have a lower HA content at the AAJ than the other test subjects. The differences between the degrees of histological fusion of the two groups may be attributed to the impact of the heat of polymerization on PMMA, the differences in the biomechanical strengths of implants, or the titanium material of APF implants.

This study has some limitations. First, experimental models were used and the sample size was small. Therefore, a statistical evaluation was not possible. Furthermore, tissue responses varied across the samples. Additional studies with more samples may be necessary to obtain more valid results. Second, we could not histologically assess AAJ fusion in the early postoperative period because we focused on comparing the outcomes of AAJ fusion using the two surgical techniques. Third, despite the AAJ having two articular surfaces, we evaluated only the ventral midline to allow for uniformity in the assessment of AAJ fusion.

Here, we performed PMF and APF on healthy beagles who underwent partial dens resection. Moreover, we evaluated AAJ fusion during postoperative observation. Our results revealed that PMF and APF resulted in AAJ stability without clinical complications, and histological findings indicating fusion were present in the area of partial resection. Therefore, PMF and APF are clinically useful fixation methods for AAI, and AAJ fixation plates made of titanium are valuable for clinical use. The AAJ fixation plate used in this study was suitable for beagles. In the future, companies should manufacture AAJ fixation plates in different sizes that are more suitable for small dog breeds that are more likely to possess naturally occurring AAI.

REFERENCES

1. Aikawa, T., Shibata, M. and Fujita, H. 2013. Modified ventral stabilization using positively threaded profile pins and polymethylmethacrylate for atlantoaxial instability in 49 dogs. *Vet. Surg.* **42**: 683–692. [Medline] [CrossRef]
2. Beaver, D. P., Ellison, G. W., Lewis, D. D., Goring, R. L., Kubilis, P. S. and Barchard, C. 2000. Risk factors affecting the outcome of surgery for atlantoaxial subluxation in dogs: 46 cases (1978–1998). *J. Am. Vet. Med. Assoc.* **216**: 1104–1109. [Medline] [CrossRef]
3. Cool, J. R. and Oliver, J. E. 1981. Atlantoaxial luxation in the dog. *Compend. Contin. Educ. Pract. Vet.* **3**: 242–250.
4. Denny, H. R., Gibbs, C. and Waterman, A. 1988. Atlanto-axial subluxation in the dog: a review of thirty cases and an evaluation of treatment by lag screw fixation. *J. Small Anim. Pract.* **29**: 37–47. [CrossRef]
5. Dickomeit, M., Alves, L., Pekarkova, M., Gorgas, D. and Forterre, F. 2011. Use of a 1.5 mm butterfly locking plate for stabilization of atlantoaxial pathology in three toy breed dogs. *Vet. Comp. Orthop. Traumatol.* **24**: 246–251. [Medline] [CrossRef]
6. Geary, J. C., Oliver, J. E. and Hoerlein, B. F. 1967. Atlanto axial subluxation in the canine. *J. Small Anim. Pract.* **8**: 577–582. [Medline] [CrossRef]
7. Genant, H. K., Block, J. E., Steiger, P., Glueer, C. C., Ettinger, B. and Harris, S. T. 1989. Appropriate use of bone densitometry. *Radiology* **170**: 817–822. [Medline] [CrossRef]
8. Hara, Y., Nezu, Y., Yasuda, H., Tuzuki, Y. and Tagawa, M. 2002. Ventral vertebral fusion technique for the dog with atlantoaxial subluxation. *Nihon Jui Masui Gekagaku Zasshi* **33**: 41–47.

9. Itoh, H., Hara, Y., Tagawa, M., Kato, T., Ochi, H., Koga, D., Okawa, A. and Asou, Y. 2012. Evaluation of the association between runt-related transcription factor 2 expression and intervertebral disk aging in dogs. *Am. J. Vet. Res.* **73**: 1553–1559. [Medline] [CrossRef]

10. Knipe, M. F., Sturges, B. K., Vernau, K. M., Berry, W. L., Dickinson, P. J., Anor, S. and LeCouteur, R. A. 2002. Atlantoaxial instability in 17 dogs. *J. Vet. Intern. Med.* **16**: 368.

11. Martinez, S. A., Arnoczky, S. P., Flo, G. L. and Brinker, W. O. 1997. Dissipation of heat during polymerization of acrylics used for external skeletal fixator connecting bars. *Vet. Surg.* **26**: 290–294. [Medline] [CrossRef]

12. McCarthy, R. J., Lewis, D. D. and Hosgood, G. 1995. Atlantoaxial luxation in dogs. *Compend. Contin. Educ. Pract. Vet.* **17**: 215–226.

13. Parry, A. T., Upjohn, M. M., Schlegl, K., Kneissl, S. and Lamb, C. R. 2010. Computed tomography variations in morphology of the canine atlas in dogs with and without atlantoaxial subluxation. *Vet. Radiol. Ultrasound* **51**: 596–600. [Medline] [CrossRef]

14. Platt, S. R., Chambers, J. N. and Cross, A. 2004. A modified ventral fixation for surgical management of atlantoaxial subluxation in 19 dogs. *Vet. Surg.* **33**: 349–354. [Medline] [CrossRef]

15. Reber, K., Bürki, A., Vizcaino Reves, N., Stoffel, M., Gendron, K., Ferguson, S. J. and Forterre, F. 2013. Biomechanical evaluation of the stabilizing function of the atlantoaxial ligaments under shear loading: a canine cadaveric study. *Vet. Surg.* **42**: 918–923. [Medline] [CrossRef]

16. Renegar, W. R. and Stoll, S. G. 1979. The use of methyl methacrylate bone cement in the repair of atlantoaxial subluxation stabilization failures. Case report and discussion. *J. Am. Anim. Hosp. Assoc.* **15**: 313–318.

17. Sanders, S. G., Bagley, R. S. and Silver, G. M. 2000. Complications associated with ventral screws, pins and polymethylmethacrylate for the treatment of atlantoaxial instability in 8 dogs. *J. Vet. Intern. Med.* **14**: 339.

18. Sanders, S. G., Bagley, R. S., Silver, G. M., Moore, M. and Tucker, R. L. 2004. Outcomes and complications associated with ventral screws, pins, and polymethyl methacrylate for atlantoaxial instability in 12 dogs. *J. Am. Anim. Hosp. Assoc.* **40**: 204–210. [Medline] [CrossRef]

19. Schulz, K. S., Waldron, D. R. and Fahie, M. 1997. Application of ventral pins and polymethylmethacrylate for the management of atlantoaxial instability: results in nine dogs. *Vet. Surg.* **26**: 317–325. [Medline] [CrossRef]

20. Seim, H. B. III. 2002. Atlantoaxial instability. pp. 1250–1255. In: Small Animal Surgery, 2nd ed. (Fossum, T. W. ed.), Mosby, St. Louis.

21. Sharp, N. J. H. and Wheeler, S. J. 2005. Atlantoaxial subluxation. pp. 161–180. In: Small Animal Spinal Disorders, 2nd ed. (Sharp, N. J. H. and Wheeler, S. J. ed.), Edinburgh, Elsevier Mosby.

22. Shires, P. K. 2003. Atlantoaxial instability. pp. 1173–1180. In: Textbook of Small Animal Surgery, 3rd ed. (Slatter, D. H. ed.), Saunders, Philadelphia.

23. Shores, A. and Tepper, L. C. 2007. A modified ventral approach to the atlantoaxial junction in the dog. *Vet. Surg.* **36**: 765–770. [Medline] [CrossRef]

24. Sorjonen, D. C. and Shires, P. K. 1981. Atlantoaxial instability: a ventral surgical technique for decompression, fixation, and fusion. *Vet. Surg.* **10**: 22–29. [CrossRef]

25. Stead, A. C., Anderson, A. A. and Coughlan, A. 1993. Bone plating to stabilize atlantoaxial subluxation in four dogs. *J. Small Anim. Pract.* **34**: 462–465. [CrossRef]

26. Takahashi, F., Hakozaki, T., Kanno, N., Harada, Y., Yamaguchi, S. and Hara, Y. 2017. Biomechanical evaluation of three ventral fixation methods for canine atlantoaxial instability: a cadaveric study. *J. Vet. Med. Sci.* **78**: 1897–1902. [Medline] [CrossRef]

27. Takahashi, F., Hakozaki, T., Kanno, N., Harada, Y., Yamaguchi, S. and Hara, Y. 2017. Evaluation of the dens-to-axis length ratio and dens angle in toy-breed dogs with and without atlantoaxial instability and in healthy Beagles. *Am. J. Vet. Res.* **78**: 1400–1405. [Medline] [CrossRef]

28. Takahashi, F., Hakozaki, T., Kouno, S., Suzuki, S., Sato, A., Kanno, N., Harada, Y., Yamaguchi, S. and Hara, Y. 2018. Epidemiological and morphological characteristics of incomplete ossification of the dorsal neural arch of the atlas in dogs with atlantoaxial instability. *Am. J. Vet. Res.* **79**: 1079–1086. [Medline] [CrossRef]

29. Takahashi, F., Hakozaki, T., Kouno, S., Suzuki, S., Sato, A., Kanno, N., Harada, Y., Yamaguchi, S. and Hara, Y. 2018. Atlantooccipital overlapping and its effect on outcomes after ventral fixation in dogs with atlantoaxial instability. *J. Vet. Med. Sci.* **80**: 526–531. [Medline] [CrossRef]

30. Thomas, W. B., Sorjonen, D. C. and Simpson, S. T. 1991. Surgical management of atlantoaxial subluxation in 23 dogs. *Vet. Surg.* **20**: 409–412. [Medline] [CrossRef]

31. van de Wijngaert, F. P. and Burger, E. H. 1986. Demonstration of tartrate-resistant acid phosphatase in un-decalcified, glycolmethacrylate-embedded mouse bone: a possible marker for (pre)osteoclast identification. *J. Histochem. Cytochem.* **34**: 1317–1323. [Medline] [CrossRef]