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Feasibility of ultrasound-guided cervical intervertebral disc injection to aid in intraoperative site identification in dog cadavers

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OBJECTIVE
To study the feasibility of ultrasound-guided intervertebral disc (IVD) injection of contrast medium and methylene blue to aid in intraoperative identification of cervical IVD spaces in dogs.

METHODS
This was a single-center experimental cadaver study using randomly chosen skeletally mature dog cadavers from January 2, 2023, to March 23, 2023. For each cadaver, 1 cervical IVD was randomly selected. The ventral cervical region was ultrasonographically examined, pushing the trachea leftwards. A 50/50 contrast medium (iohexol, 300 mgI/mL) and methylene blue mixture was injected under ultrasound guidance in the IVD and ventral soft tissues. The x-rays and CT scans were performed before and after injection. Each cadaver was dissected using a ventral slot procedure. The cadavers and IVD characteristics, the success rate in ultrasonographically identifying the correct IVD space, time to injection, most caudal IVD space feasibly injectable, and semiquantitative imaging and surgical scores were recorded.

RESULTS
20 canine cadavers were used. The IVD injections were successfully performed in the correct IVD space in all cadavers. The median time to injection was 3.95 minutes (Q1 to Q3, 3.22 to 5.88 minutes). The contrast medium was clearly visible on at least 1 radiographic projection in all but 1 case and on the CT in all cases. During surgical dissection, the dye was clearly visible in all but 1 case. The dogs' weight was significantly different between most caudal IVD spaces feasibly injectable.

CONCLUSIONS
Ultrasonographical cervical IVD space identification and mixture injection are feasible and might help intraoperative cervical IVD space identification in dogs.

CLINICAL RELEVANCE
This technique could be used presurgically in patients with cervical IVD diseases.

Keywords: ultrasound, cervical intervertebral disc, injection, dog, guidance system

Intervertebral disc (IVD) herniation commonly affects dogs and may require surgery. Diagnosis is based mostly on clinical exam and on advanced imaging modalities for the precise localization of the lesion.[1] Intraoperative orientation for cervical IVD herniations is predominantly based on digital palpation, which can be misleading and result in mistakes.

Wrong-level spine surgery is rarely reported with variable consequences for the patient, owner, and surgeon including medical problems, financial implications, and psychological effects.[2–10] In human literature, 1 study[2] reported the incidence of wrong-level surgeries in the cervical spine to be 0.068%, with 16.0% of responding surgeons having made this mistake at least once. Another study demonstrated 0.03% of wrong-level spinal surgeries, 21.0% of which were in the cervical region, and 50.0% of responding

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surgeons performed 1 or more wrong-level spinal surgeries, 21.0% of which were in the cervical region.[3] In a recent study,[10] 47.5% of surgeons had been involved in wrong-level spinal surgery. In veterinary medicine, a study[9] concerning thoracolumbar disc herniations reported 1.28% of wrong-level surgeries. Although the prevalence of wrong-level surgeries in the cervical spine has not been described in veterinary literature, multiple anatomical variations affecting the cervical region and therefore the anatomical landmarks used by surgeons have been described in dogs. These include block vertebrae, C7 transitional vertebrae (cervical ribs), asymmetry of C6, and anatomical variation of neck muscles. All could increase the risk of wrong-level surgeries.[11–14]

To avoid such situations, and depending on the surgeon's preference, techniques exist to facilitate IVD localization, including imaging and guidance systems placed preoperatively.[2–10,15,16] Preoperative imaging includes ultrasonography whose main advantages are wide availability, portability, and absence of ionizing radiations.[9,15,16] It may however be technically challenging for inexperienced users and warrant the assistance of a radiologist during the surgery. Intraoperative fluoroscopy or radiography is often considered the gold standard but is not widely available and exposes users to ionizing radiations.[2–10,15–20] Preoperative guidance systems include marking a spinous process and regional soft tissues using methylene blue or placing a needle within a spinous process using a radiographic or radioscopic device.[6,21] These systems are widely used in cases of thoracolumbar IVD herniations for which a hemilaminectomy is commonly performed.[21] In cases of cervical IVD herniations, a ventral slot procedure is commonly performed. Due to the dorsal recumbency of the patient and the at-risk anatomical structures located in the ventral aspect of the cervical region, the previously described guidance techniques are not possible. The use of ultrasound (US) guidance for cervical intradiscal injections, enabling the manipulator to avoid major regional structures without using ionizing radiations, has previously been described in human literature but is lacking in dogs.[16] To the author's knowledge, there is only 1 cadaveric study[22] describing the use of US guidance for canine cervical IVD injections. It also compared it to fluoroscopy and CT scan. The precise technique and approach used for US guidance were however not described, therefore limiting repeatability.

The aims of this study were to verify the feasibility of preoperative US-guided injection of a mixture of contrast medium and methylene blue within the ventral aspect of cervical IVD and the ventral soft tissues (mainly the longus colli muscle) using a midline approach to aid in intraoperative surgical site identification and to assess if cadaver and IVD characteristics influence the success of the technique. We also estimated the most caudal IVD feasibly injectable. We hypothesized that the injection technique would be feasible without damaging regional structures, that a postinjection radiographic study would enable contrast medium visualization to confirm the correct location of the injection, and that methylene blue would be visualized during surgical dissection, therefore confirming the correct IVD identification.

Methods

Study design

The study was a single-center experimental cadaver study. Upon admission of the patients to the clinic, owners signed a consent form included within the hospitalization contract.

Animals

The study was performed on canine cadavers with full clinical histories and without any history or clinical signs attributable to the cervical spine region. The dogs were naturally deceased or euthanized for reasons unrelated to the study. The sample size was based on the availability of a single institution from January 2, 2023, to March 23, 2023. Cadavers were excluded from the study in cases of juvenile patients and if the anatomy of the cervical region was altered. Breed, sex, neutered status, age, weight, and body condition score (BCS) were noted. Cadavers were also divided into 3 different weight groups: < 10 kg, 10 to 30 kg, and > 30 kg.

Study protocol

Preinjection imaging

Before injection, plain orthogonal radiographs (laterolateral and ventrodorsal projections) of the cervical spine were acquired for each cadaver using a direct radiography system. Acquisition parameters used depended on the size of the dog as in a clinical setting (ranges, 50 to 85 kVp and 6.3 to 25 mAs). A plain CT scan of the cervical region was also acquired for each cadaver with the patient placed in dorsal recumbency and the thoracic limbs flexed caudally along the body of the dog. Acquisition parameters used depended on the size of the dog (range, 120 to 140 kV and 250 to 300 mAs; pitch factor = 0.6). Scan tube current was modulated by automatic exposure control. Image data sets were reconstructed using parameters of 120-mm field of view, 512 X 512 matrix, 0.6- to 1-mm slice thickness, and a spine reconstruction algorithm (window level, 40; window width, 350) as well as a bone reconstruction algorithm (window level, 700; window width, 4,000) with iterative reconstruction. All images were stored in a DICOM format on a local Picture Archiving and Communication System. They were reviewed by a European College of Veterinary Diagnostic Imaging resident (JF) in an image viewing program. The presence of any relevant abnormalities affecting the injected IVD, IVD spaces, and the adjacent vertebrae was characterized and graded using a semiquantitative scoring system. The absence (0/2) or presence of spondylosis on either one epiphysis (1/2) or both epiphyses (2/2) adjacent to the injected disc was noted. The presence (1/1) or absence (0/1) of mineralization of the injected IVD was also checked. It was also noted if the injected IVD space was decreased in size (1/1) or not (0/1). Finally, these different scores were added up to obtain an IVD score, ranging from 0/4 to 4/4 and representing the grade of lesions affecting the injected IVD and adjacent vertebral epiphyses, with 4/4 being the highest level of lesions **(Figure 1)**.

Ultrasound-guided injection

For each cadaver, 1 IVD space (C2-C3 to C6-C7) was randomly selected (target site). A 1-mL syringe containing a mixture (50/50 volume) of iodinated nonionic contrast medium (iohexol, 300 mgI/mL) and methylene blue and mounted with a 22-G X 2-inch 0.7 X 50-mm needle was prepared for each cadaver. The total injectable volume was 0.2 mL for 50.0% of the cadavers and 0.3 mL for the remaining 50.0%. For the US examination, a fixed US machine equipped with a high-frequency (4.5 to 18 MHz) linear probe was used. The dogs were placed in dorsal recumbency, and the ventral aspect of the whole cervical region was clipped as it would be for a ventral slot surgery preparation. The neck was maintained in extension and straight by a second person. Alcohol was used as a coupling agent. The probe was placed on the skin by a single operator (JF), parallel to the

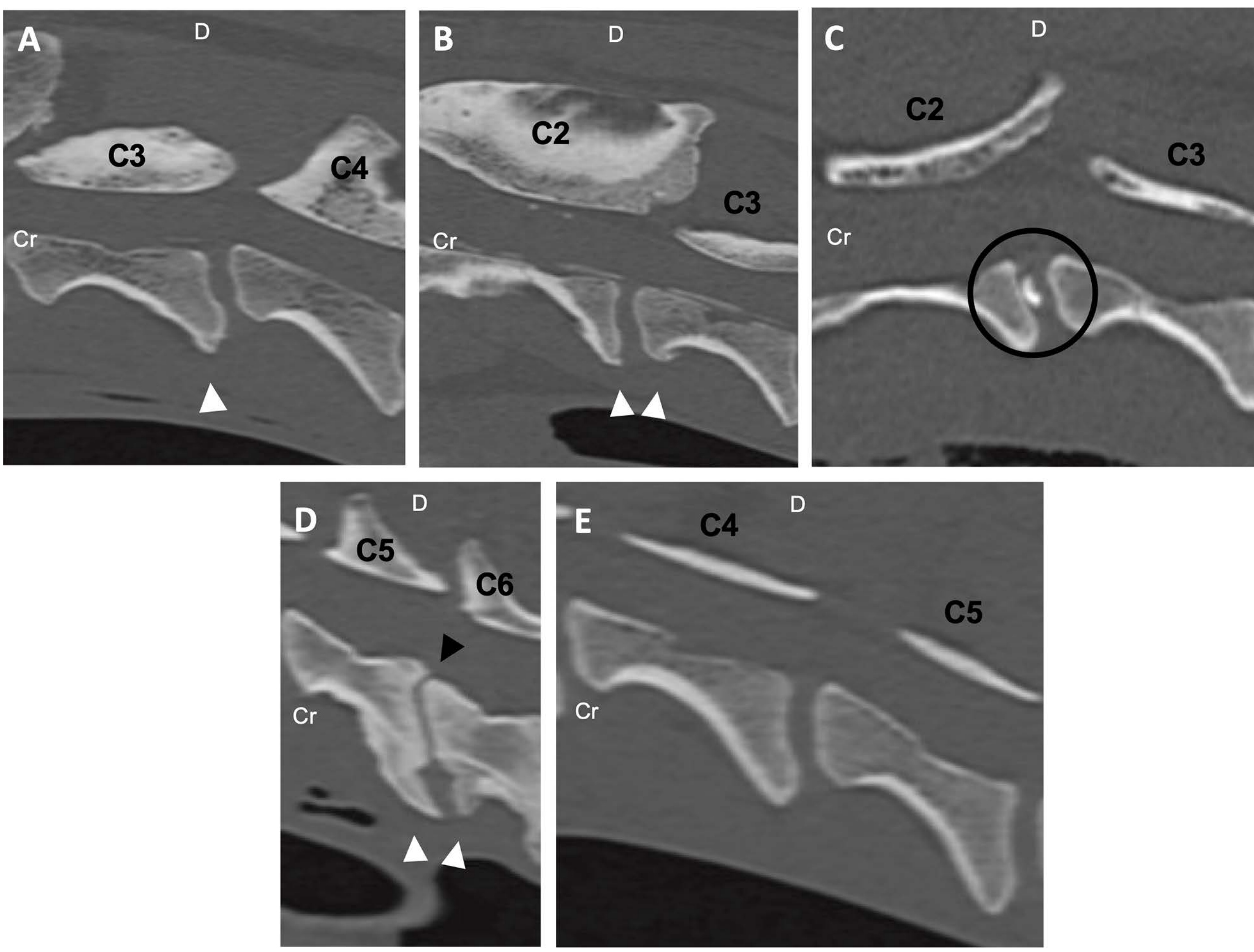

Figure 1—Sagittal midline plane reconstruction CT images of the cervical spine of 5 different canine cadavers, reconstructed using parameters of 120-mm field of view, 512 X 512 matrix, 0.6- to 1-mm slice thickness, and a bone reconstruction algorithm (window level, 700; window width, 4,000) with iterative reconstruction, acquired before the ultrasound (US)-guided injection of a mixture of methylene blue and contrast medium (iohexol, 300 mgI/mL) within a target intervertebral disc (IVD) and the ventral soft tissues. This was performed on a total of 20 randomly selected canine cadavers of various breeds, sexes, neutered status, ages, weights, and body condition scores, with full clinical histories, without any history or clinical signs attributable to the cervical spine region, naturally deceased or euthanized for reasons unrelated to the study. The sample size was based on the availability in a single institution during the period from January 2, 2023, to March 23, 2023. Cadavers were excluded from the study in cases of juvenile patients or if the anatomy of the cervical region was altered. These images were used to evaluate the relevant abnormalities affecting the injected IVD using a modified semiquantitative scoring system called the IVD score. This score (/4) is the sum of 3 subscores corresponding to relevant abnormalities affecting the injected IVD, IVD spaces, and adjacent vertebrae including the following: spondylosis (white arrowheads): presence on either one epiphysis (1/2) (A) or both epiphyses (2/2) (B and D) adjacent to the injected IVD or absence (0/2) (C and E); mineralization of the injected IVD (black circle): presence (1/1) (C) or absence (0/1) (A, B, D, and E); and decrease in size of the injected IVD space (black arrowhead): yes (1/1) (D) or no (0/1) (A, B, C, and E). If none of these abnormalities were present, the IVD was given an IVD score of 0/4 (E). Window width, 4,000 HU; window level, 700 HU; 0.6-mm slice thickness. Cr = Cranial. D = Dorsal.

long axis of the neck and at the level of the median plane of the cervical vertebrae, by pushing the trachea and other regional anatomical structures to the left with the use of the probe to enable a safe access route for the injection. The region was then examined from C1 to the target site. The main landmarks used to confirm initial location were (1) the body of C1, which is shorter than the rest of the vertebral bodies; (2) the wings of C1, which are clearly visualized as a prolongation of the hyperechoic interface of the vertebral body, when making a laterolateral movement with the probe from the body and giving acoustic shadowing more laterally than the other vertebrae; and (3) the intervertebral space between C1 and C2, which lacks an IVD. Each intervertebral space was counted by the operator under the supervision of a European College of Veterinary Diagnostic Imaging board-certified radiologist (GB) until the targeted IVD. The transverse processes of C6, which are notably larger than those of the adjacent vertebrae and give larger acoustic shadowing, could be used as an additional landmark for confirmation of location if needed **(Figure 2; Supplementary Video S1)**. Once the correct intervertebral space was identified, the mixture was injected in the ventral aspect of the IVD and in the soft tissues (mainly the longus colli muscle) located ventrally in the IVD space. The needle was inserted cranially to the probe in the plane of the US beam and angled cranioventral-caudodorsally toward the IVD space until it penetrated the IVD while making sure to avoid any at-risk regional structures, and then slowly pulled out after starting the injection of the mixture **(Figure 3; Supplementary Video S2)**. After the injection was performed and the needle removed, the operator estimated visually the most caudal IVD space that could be feasibly injected with this technique (C5-C6, C6-C7, or C7-T1). The time to injection was recorded from the moment the probe was placed on the skin until the end of the injection.

Postinjection imaging

After the US-guided injection of the mixture, x-rays and a CT scan were identically performed on each cadaver as before the injection, with the CT scan considered the gold standard. Images were reviewed and characterized using a semiquantitative

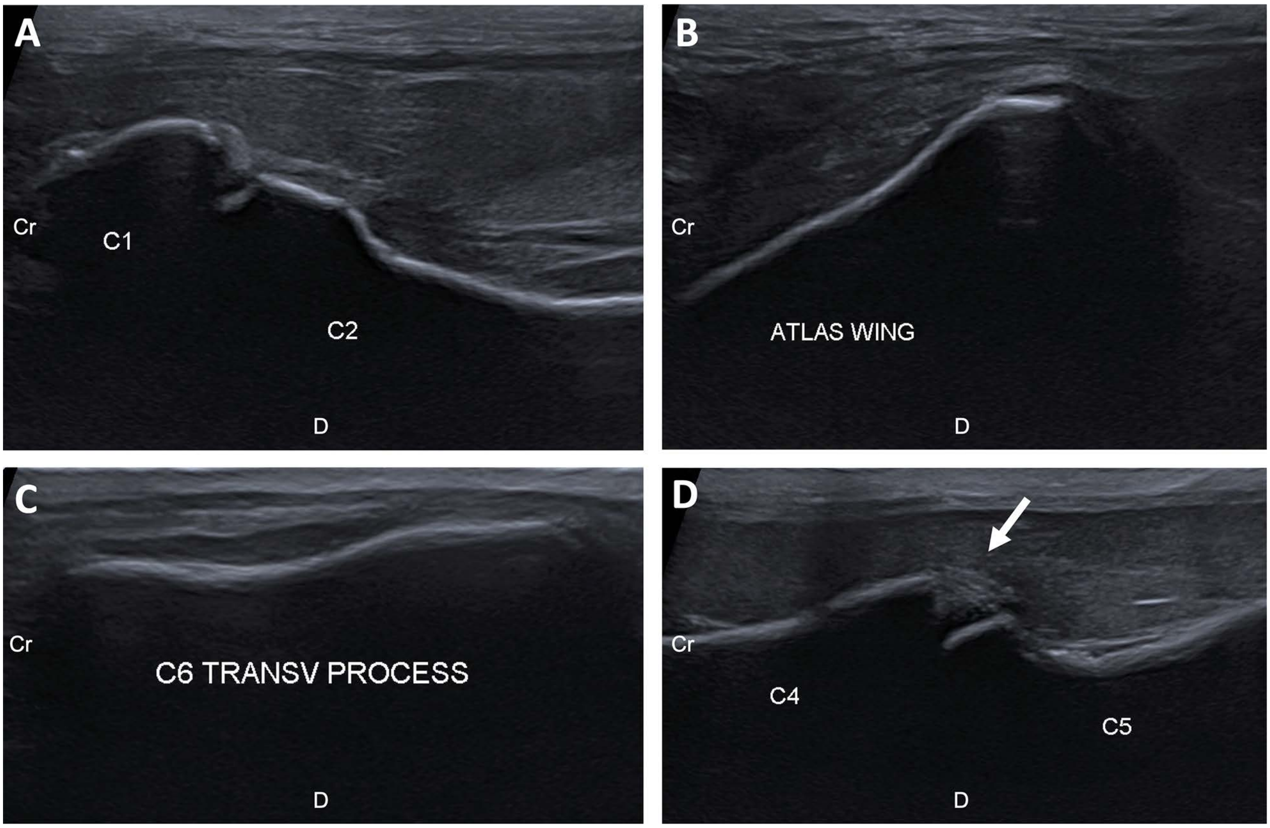

Figure 2—Ultrasound examination of the ventral aspect of the cervical region of a canine cadaver belonging to the same study population as described (Figure 1). The probe was placed parallel to the long axis (longitudinal plane) and on the median plane of the cervical vertebrae while pushing the trachea and other regional anatomical structures to the left. The region was examined from C1 to the targeted site by counting each intervertebral space. The body and wings of the atlas (C1); the intervertebral space between C1 and C2, which lacks an IVD; and the transverse processes of C6 (C6 TRANSV PROCESS) were used as anatomical landmarks to confirm location. A—Ventral aspect of the atlas (C1), which has a shorter body compared to other vertebrae, and of the axis (C2) in a median plane; the absence of an IVD between C1 and C2 can be noted. B—Ventral aspect of a wing of the atlas (C1). C—Ventral aspect of the transverse process of C6. D—Ventral aspect of C4, C5, and the IVD between the 2 vertebrae (white arrow) in a median plane.

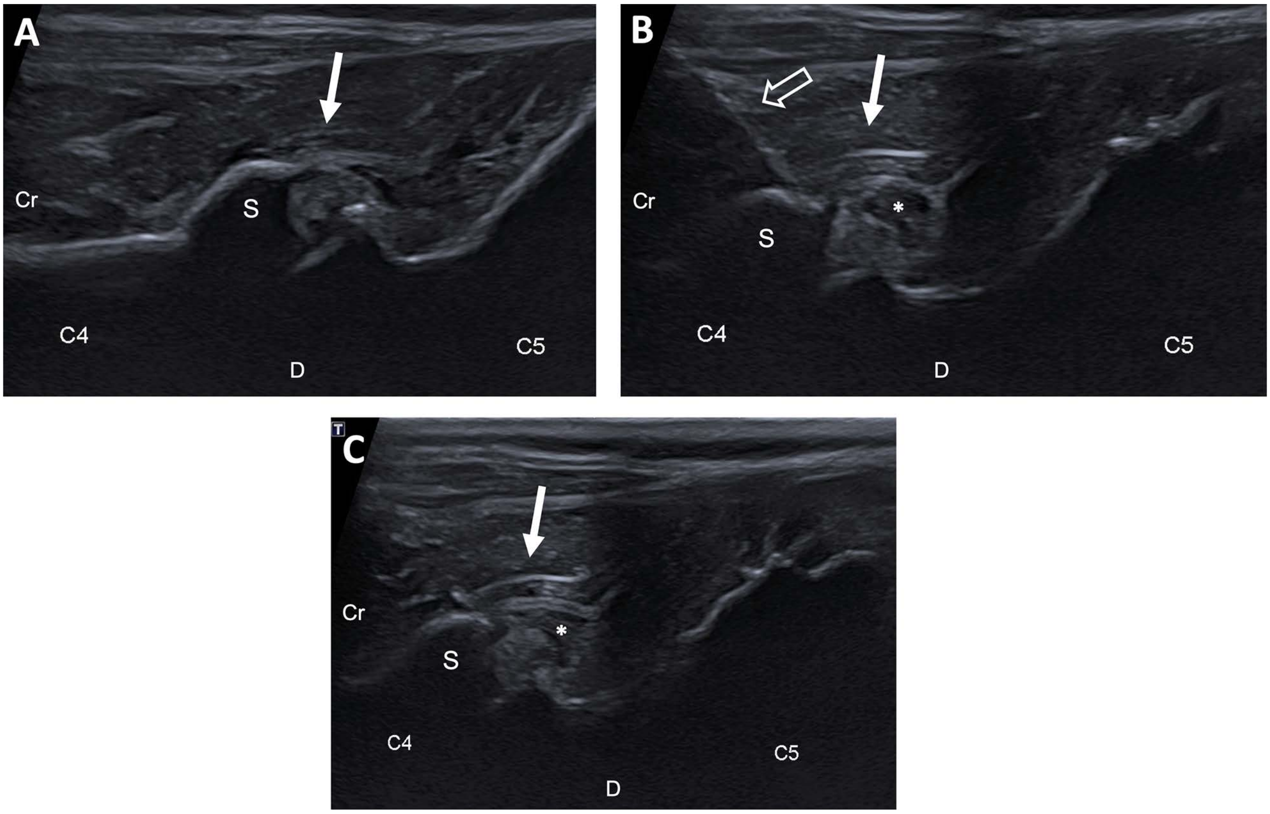

Figure 3—Ultrasound examination of the ventral aspect of C4 and C5 and the IVD between the 2 vertebrae (white arrows) before (A), during (B; with the needle visualized to the left of the image [white outlined arrow]), and after (C) the injection of the mixture within the IVD and the ventral soft tissues of a canine cadaver belonging to the same study population as described (Figure 1). The probe was placed in a longitudinal and median plane while pushing the trachea and other regional anatomical structures to the left. A large amount of new bone formation/spondylosis (S) can be noted on the caudal epiphysis of C4. In (B) and (C), a hypoechoic region (asterisks) corresponds to the injectate located within the IVD and ventral soft tissues, which are bulging ventrally compared to (A).

scoring system by a single operator (JF). First, the success rate in ultrasonographically identifying and injecting the correct IVD based on the postcontrast CT scan images was recorded, applying a simple binary score: successful (1/1) or unsuccessful (0/1).

A semiquantitative scoring system was used to characterize the x-rays and CT scan images. For the x-ray scoring, images were anonymized and randomized by one of the coauthors (KG) who was blinded to the manipulations. The blinded operator (JF) was then given the 40 anonymized and randomized x-rays (20 in laterolateral projection and 20 in ventrodorsal projection) and asked to give a score out of 2 to characterize the conspicuity of the contrast medium. The scoring was applied as such 0/2 meant that the contrast medium was not visible or that there was an error in the recognition of the injected IVD space, 1/2 meant that the injected IVD space was correctly recognized but that the contrast medium was unclearly visible or that the images were doubtful, and 2/2 meant that the injected IVD was correctly recognized with clear visualization of the contrast medium. For each cadaver, the score of the laterolateral projection was added to the score of the ventrodorsal projection, giving therefore a global x-ray conspicuity score out of 4, with 4/4 corresponding to the highest possible conspicuity level **(Figure 4)**.

For the CT scan scoring, variable elements were analyzed. The presence (1/1) or absence (0/1) of contrast medium marking the IVD was evaluated. The marking of the soft tissues located ventrally to the injected IVD by the contrast medium was also characterized. A score of 1/1 was given if the contrast medium was visualized within the soft tissues located on the median plane of the IVD (mainly the longus colli muscle), and a score of 0/1 was given if not. A score of 1/1 was also given if the contrast medium was visualized within the soft tissues located paramedianly to the IVD, and a score of 0/1 was given if not. A higher score would therefore mean a better marking of the soft tissue, presumably meaning a better identification of the IVD space during surgery. The operator also noted the extension of the contrast medium within the soft tissue beyond the level of the mid body of the vertebrae located cranially and/or caudally to the injected IVD; a score of 0/1 was given if such was the case, representing a possible cause of confusion during surgery, and a score of 1/1 was given if not meaning that the marking was located relatively focally relative to the injected IVD. These 4 scores were then added up,

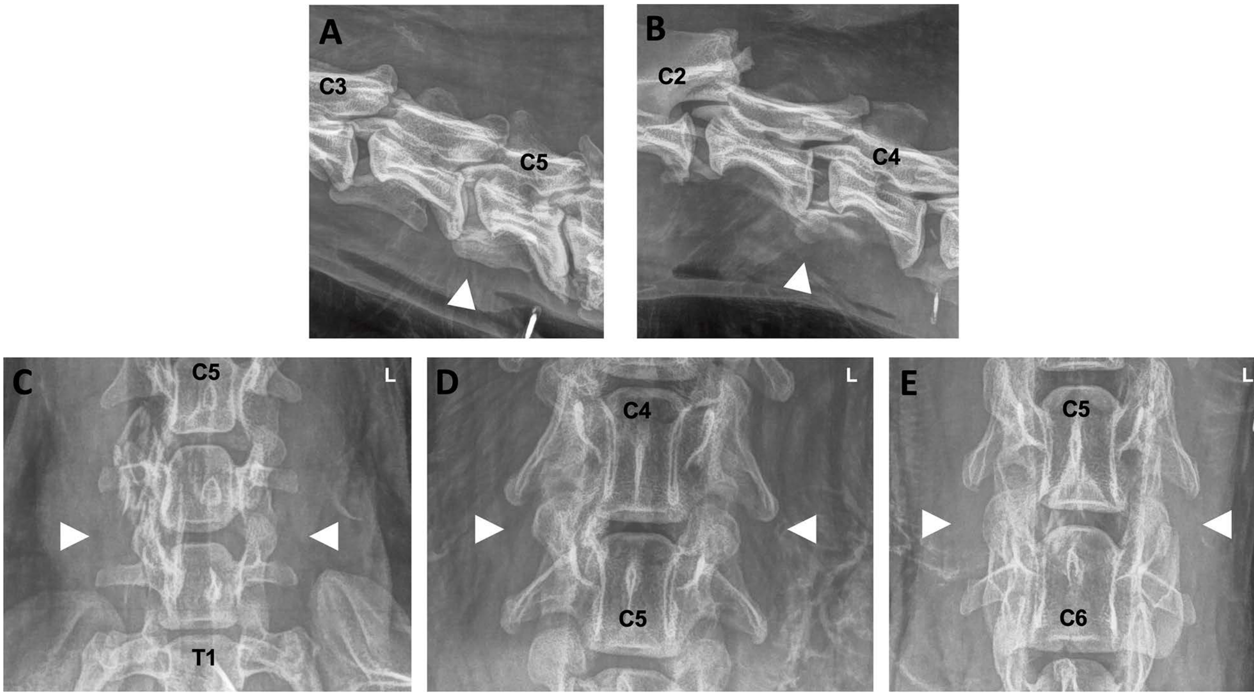

Figure 4—Left-right lateral (A and B) and dorsoventral (C–E) radiographic views of the cervical spine of 5 different dogs, belonging to the same study population as described (Figure 1), after US-guided injection of the mixture within the target IVD and ventral soft tissues (white arrowheads). These images were used to evaluate a semiquantitative scoring system called the x-ray conspicuity score. This score (/4) is the sum of 2 subscores corresponding to the conspicuity of the contrast medium on both radiographic views. A subscore of 0/2 was given if the contrast medium was not visible or if there was an error in the recognition of the injected IVD space (C). No lateral radiograph received this subscore. A subscore of 1/2 was given if the injected IVD space was correctly recognized but the contrast medium was unclearly visible or the images were doubtful (A and D). A subscore of 2/2 was given if the injected IVD was correctly recognized with clear visualization of the contrast medium (B and E). L = Left.

giving a global CT conspicuity score out of 4, representing the conspicuity of the contrast medium injection on CT and therefore defining the quality of the injection, with 4/4 representing the highest possible quality **(Figure 5)**.

Surgical dissection

Each cadaver was then dissected by a European College of Veterinary Surgeons board-certified surgeon (PP) as if performing a ventral slot procedure until visualization of the ventral aspect of the annulus fibrosus of the target IVD. In hopes of limiting a possible confirmation bias, the surgeon knew the target IVD but was unaware of the success or failure of the US-guided injection. The time period between the injection and the surgical dissection varied from a few hours to 24 hours. The target IVD was identified by manual palpation, as per usual for this procedure, and the visibility of the methylene blue marking was evaluated and scored using a semiquantitative scoring system (surgical score). A score of 2/2 was given if the methylene blue was clearly and undoubtedly visible, a score of 1/2 was given if the marking was unclearly visible or confusing, and a score of 0/2 was given if the methylene blue was not visible or led to a mistake in the identification of the target IVD. During surgical dissection, any damage to the regional structures (punction, laceration, etc) was evaluated and given a score of 1/1 if present or of 0/1 if absent.

Statistical analysis

The studied variables for each patient included the dog's characteristics (age, sex, breed, weight, weight group, and BCS), injected IVD, IVD lesion characterization (IVD score), quantity of mixture injected, time between the beginning of the US exam and the injection, success rate in ultrasonographically identifying and injecting the correct IVD, most caudal IVD feasibly injectable, x-ray conspicuity score, CT conspicuity score, and surgical score. All considered variables were described using appropriate descriptive statistics: frequency table and associated percentage were presented for qualitative variables, while median and IQR (Q1 to Q3) were assessed for quantitative variables. The normality of the distribution of the quantitative variables was verified numerically and graphically; the Shapiro-Wilk normality test completed this investigation of the normality. Univariate linear models were conducted to model the timing between the beginning of the US exam and the injection in the function of all considered dogs' and IVD characteristics. To verify the normality and homogeneity of the residuals, a log transformation was applied to the dependent variable. The conspicuity score on laterolateral x-ray evaluation was compared to the conspicuity score on ventrodorsal x-ray evaluation using a symmetry test. The impact of all considered characteristics on the conspicuity score based on x-ray evaluation was analyzed

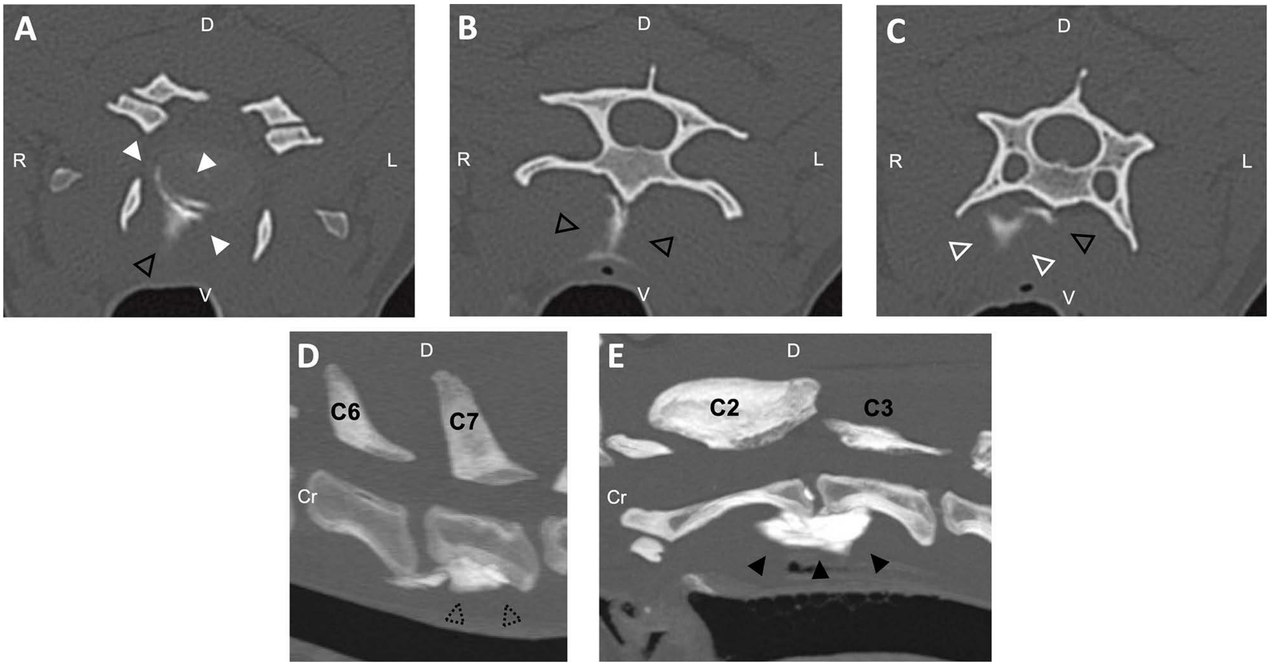

Figure 5—Transverse plane (A–C) reconstruction CT images of the C3-C4 IVD space (A), the caudal aspect of C3 (B), and the cranial aspect of C4 (C) of a dog. Sagittal midline plane (D and E) reconstruction CT images of the cervical spine of a second dog from C6 to C7 (D) and of a third dog from C2 to C3 (E) with maximum intensity projection. All 3 dogs belonged to the same study population as described (Figure 1). All CT images used a bone reconstruction algorithm and were acquired after the US-guided injection of the mixture within the IVD and ventral soft tissues. These images were used to evaluate a semiquantitative scoring system called the CT conspicuity score. This score (/4) is the sum of 4 subscores corresponding to the characterization of the contrast medium injection on CT and therefore defining the quality of the injection. The subscores included presence of contrast medium marking the IVD: yes (1/1) (A; white arrowheads) and no (0/1); presence of the contrast medium within the soft tissues located on the median plane ventrally to the injected IVD (mainly the longus colli muscle): yes (1/1) (A–C; black outlined arrowheads) and no (0/1); presence of the contrast medium within the soft tissues located on the paramedian plane ventrally to the injected IVD: yes (1/1) (C; white outlined arrowheads) and no (0/1); and extension of the contrast medium within the soft tissue beyond the level of the mid body of the vertebrae located cranially and/or caudally to the injected IVD: yes (0/1) (D; black dotted arrowheads) and no (1/1) (E; black arrowheads). Window width, 4,000 HU; window level, 700 HU; 0.6-mm slice thickness. R = Right. V = Ventral.

using the Fisher exact test for qualitative variables and using Mann-Whitney or Kruskal-Wallis tests for quantitative variables. The same method was used to analyze the conspicuity score obtained for each x-ray evaluation and conspicuity score based on CT evaluation as well as the impact of dogs' characteristics on the most caudal IVD space feasibly injectable. Statistical significance was assessed at a 5.0% level (P < .05). Analyses were performed only on observed data; missing data were not replaced. The statistical software packages used were SAS, version 9.4 (SAS Institute Inc), and R, version 4.2 (The R Foundation).

Results

Twenty canine cadavers of various breeds and sizes were used. The dogs had a median age of 7.0 years (Q1 to Q3, 4.5 to 13.0 years) and a median body weight of 22.7 kg (Q1 to Q3, 9.1 to 36.9 kg) with a median BCS of 5/9 (Q1 to Q3, 4 to 6.5). They were divided into 3 different weight groups: < 10 kg (25.0%; median weight, 6.8 kg; Q1 to Q3, 4.85 to 9.05 kg), 10 to 30 kg (40.0%; median weight, 20.2 kg; Q1 to Q3, 18.2 to 25.5 kg), and > 30 kg (35.0%; median weight, 41.0 kg; Q1 to Q3, 35.7 to 42.4 kg). The breeds included 4 mixed-breed dogs as well as 1 of each of the following breeds: Border Collie, German Shepherd, Beagle, Bouvier des Flandres, Siberian Husky, Chihuahua, Rottweiler, Samoyed, Yorkshire Terrier, Weimaraner, Bernese Mountain Dog, Leonberg, Alpenländische Dachsbracke, Belgian Shepherd, Bichon, and Poodle. There were 25.0% entire males, 30.0% castrated males, 10.0% entire females, and 35.0% spayed females.

Abnormalities affecting the injected IVD were spondylosis on one of the epiphyses adjacent to the target IVD in 5/20 (25.0%) dogs and on both epiphyses in 5/20 (25.0%) dogs, disc degeneration/mineralization in 5/20 (25.0%) dogs, and decrease in size of the IVD space in 1/20 (5.0%) dogs. IVD scores were as follows: 0/4 in 9/20 (45.0%) dogs, 1/4 in 5/20 (25.0%) dogs, 2/4 in 3/20 (15.0%) dogs, 3/4 in 2/20 (10.0%) dogs, and 4/4 in 1/20 (5.0%) dogs.

Of the dogs, 4/20 (20.0%) were injected in C2-C3, 4/20 (20.0%) were injected in C3-C4, 4/20 (20.0%) were injected in C4-C5, 4/20 (20.0%) were injected in C5-C6, and 4/20 (20.0%) were injected in C6-C7.

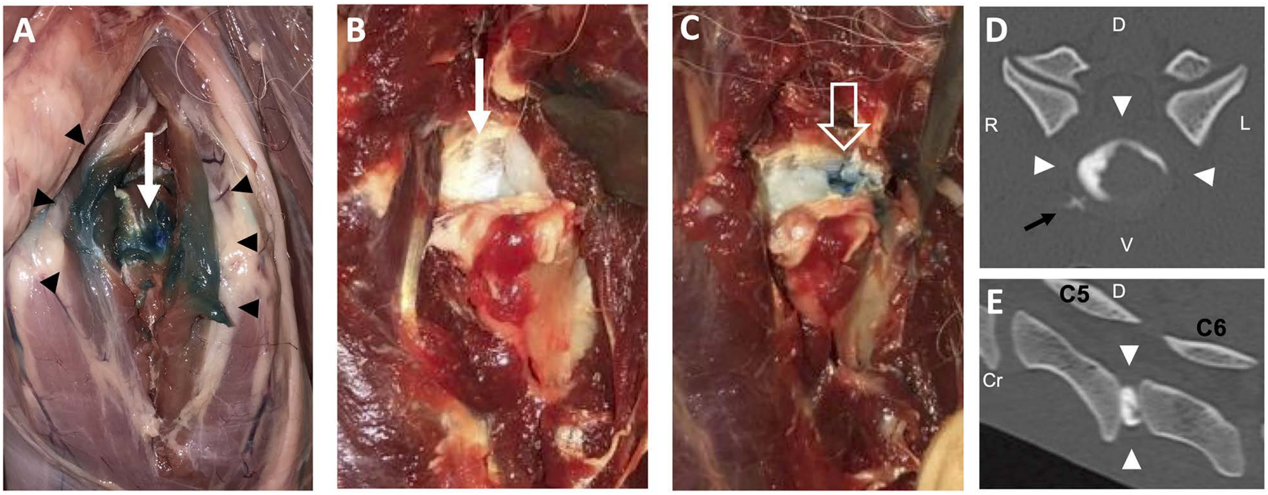

Figure 6—Photographs (A–C) of the ventral aspect of the cervical region of 2 different canine cadavers (A: first cadaver; B and C: second cadaver), belonging to the same study population as described (Figure 1), after injection of the mixture within the IVD (A and C) and within the ventral soft tissues (A). Transverse plane reconstruction CT image of the C5-C6 IVD space (D) and sagittal midline plane reconstruction CT image of C5 and C6 and of the IVD space between the 2 vertebrae (E) of the second cadaver (B and C). Both CT images were acquired after the US-guided injection of the mixture and used a bone reconstruction algorithm. Each cadaver was dissected by a European College of Veterinary Surgeons board-certified surgeon (PP) as if performing a ventral slot procedure, using manual palpation for target IVD identification, until visualization of the ventral aspect of the target IVD (A and B; white arrows). The ventral aspect of the IVD of the second cadaver was further dissected until visualization of the methylene blue, which was injected within the IVD (white outlined arrow) and not in the ventral soft tissues along the dissection path (C). During surgical dissection, a quantitative scoring system named surgical score was used to characterize the visibility of the methylene blue and therefore evaluate the quality of the injection. A score of 2/2 was given if the methylene blue was clearly and undoubtedly visible as in the first cadaver (A) in which the soft tissues (black arrowheads) and ventral aspect of the IVD (white arrow) are marked. A score of 1/2 was given if the marking was unclearly visible or confusing as in the second cadaver (B and C) in which the soft tissues along the dissection path were not marked and the methylene blue was seen only after surgical dissection of the ventral aspect of the annulus fibrosus (C; white outlined arrow). The CT images of the second cadaver confirm that the contrast medium is mostly visualized within the IVD (D and E; white arrowheads), that only a very small amount marked the paramedian ventral soft tissues (along the fascias between the longus colli and intertransversarii ventralis cervicis muscles) (E; black arrow), and that none marked the median ventral soft tissues (notably the longus colli muscle). A score of 0/2 was given if the methylene blue was not visible or led to a mistake in the identification of the target IVD. No case in this study received this score.

The IVD injections were successfully performed in the target IVD in all cadavers (20/20). The time to injection was recorded for all but 1 case. The median time to injection was 3.95 minutes (Q1 to Q3, 3.22 to 5.88 minutes). None of the parameters tested had any statistical effect on the injection time, including the IVD score ($P > .05$).

The most caudal IVD space feasibly injectable was C5-C6 in 1/20 (5.0%) cadavers with a weight of 41.0 kg and a BCS of 9/9, C6-C7 in 7/20 (35.0%) cadavers with a median weight of 34.0 kg (Q1 to Q3, 25.0 to 41.0 kg) and a median BCS of 6 (Q1 to Q3, 4 to 7), and C7-T1 in 12/20 (60.0%) cadavers, with a median weight of 13.7 kg (Q1 to Q3, 7.3 to 23.0 kg) and a median BCS of 4 (Q1 to Q3, 3.5 to 5.5). Injection was therefore estimated feasible for all of the IVD from C2-C3 to C6-C7 in 19/20 (95.0%) cases. Dogs' weight was significantly different between most caudal IVD spaces feasibly injectable ($P = .028$); the median weight was significantly lower for C7-T1 IVD than for C6-C7 IVD.

The conspicuity of the contrast medium on laterolateral x-rays was unclear in 4/20 (20.0%) cases and clear in 16/20 (80.0%) cases. On ventrodorsal x-rays, the contrast medium was not visible/there was an error in the recognition of the injected IVD space in 6/20 (30.0%) cases, the contrast medium was unclearly visible in 6/20 (30.0%) cases and clearly visible in 8/20 (40.0%) cases. Also, the contrast medium was clearly visible on at least 1 of the 2 radiographic projections in all but 1 dog. The x-ray conspicuity score was 4/4 in 5/20 (25.0%) dogs, 3/4 in 9/20 (45.0%) dogs, 2/4 in 5/20 (25.0%) dogs, and 1/4 in 1/20 (5.0%) dogs. There was no statistically significant difference in the conspicuity of the contrast medium on x-rays between the injected volumes of the mixture ($P = .50$). There was no statistically significant difference between the IVD score and the x-ray conspicuity score ($P = .20$).

On CT scan examination, the IVD was marked in all dogs (20/20). The ventral soft tissues located on the median plane of the IVD (mainly the longus colli muscle) were marked by the contrast medium in 17/20 (85.0%) dogs, and those located on the paramedian plane of the vertebrae were marked in 20/20 (100.0%) dogs. The contrast medium extended beyond the mid body of the vertebrae cranially and/or caudally to the injected IVD in 13/20 (65.0%) dogs, 9 of which were injected with 0.3 mL of mixture and 4 of which were injected with 0.2 mL. The CT conspicuity score was 4/4 in 6/20 (30.0%) dogs, 3/4 in 12/20 (60.0%) dogs, and 2/4 in 2/20 (10.0%) dogs. The IVD score was significantly different according to the CT conspicuity score (P = .0015); higher CT conspicuity scores were observed in cases with lower IVD scores and therefore had fewer changes in the target IVD, IVD space, and surrounding vertebrae. Other characteristics did not differ according to the CT score or vice versa.

During surgical dissection, the methylene blue was visualized in all cadavers (20/20). It was clearly visible in 19/20 (95.0%) dogs and unclearly visible in 1/20 (5.0%) dogs. In the latter case, the vast majority of the mixture was injected within the IVD, and only a very small amount marked the paramedian ventral soft tissues (along the fascias between the longus colli and intertransversarii ventralis cervicis muscles), while none marked the median ventral soft tissues (notably the longus colli muscle; **Figure 6**). The at-risk regional structures were inspected during the surgical dissection by the surgeon, and no damage (punction, laceration, etc) was identified.

Discussion

The results of a US-guided injection of a methylene blue and contrast medium mixture within IVD and ventral soft tissues (mainly the longus colli muscle) are satisfying. The injections were successfully performed in the correct IVD in all cases, the contrast medium was visualized clearly in at least 1 of 2 radiographic projections in all but 1 case and on the CT scan in all cases, and the methylene blue was visualized clearly during surgical dissection in all but 1 case. There was no visible damage to the regional structures during surgical dissection. The median time to perform the injection was 3.95 minutes (Q1 to Q3, 3.22 to 5.88 minutes).

Ultrasound-guided percutaneous injection of canine cervical IVD is very scarcely described in veterinary literature. A similar technique was used to inject a therapeutic agent within IVD in dogs.[22] The technique was however not precisely described, which limited repeatability. The study population included mostly small dogs (10/12), and the necropsy technique did not mimic a ventral slot procedure, which was crucial in this study to verify the usefulness of the technique for cervical herniation surgeries. The success rate of cervical IVD injection using US guidance (43.0%) was inferior compared to fluoroscopy (56.0%) and CT guidance (100.0%). This varied from our results in which the success rate was 100.0%. However, the authors considered an injection successful only if the nucleus pulposus was marked, while we did not characterize the IVD markings, therefore limiting the comparison between the studies. Another veterinary study[9] found the US to have a success rate of 96.7% for identifying target thoracolumbar IVD, which is similar to our results in the cervical region.

The US-guided percutaneous intradiscal injection through a ventral approach used in our study is similar to the midline approach described in human literature as that approach also used tracheal deviation, although a finger was used instead of the US probe.[16] The techniques also differ in the fact that our US probe was placed longitudinally compared to the axis of the vertebrae, while the US probe was placed transversely in the other study. We found that the longitudinal placement of the probe facilitated the identification of the different vertebrae and IVD. Safety zones have been described to avoid at-risk structures during percutaneous cervical approach in humans.[23] Such measurements were not made in our study, but we followed the same methodology to avoid these structures. Another study[16] in human literature placed the patients in a lateral decubitus allowing gravity to shift the regional at-risk structures, adding to the manual deviation. In our study, patients were placed in dorsal recumbency with the shifting of the regional at-risk structures relying only on manual deviation, which we found to be sufficient.

The additional time taken for the procedure could be seen as negative. However, we speculated that the duration of surgery could be reduced once the IVD and soft tissues are marked as the surgeon would confidently and rapidly locate the target IVD space. Furthermore, this procedure would remove the necessity of postoperative imaging currently needed when surgeons doubt the operated IVD and therefore decrease the total time of anesthesia. Finally, the added time of the procedure would be insignificant compared to the additional time and various implications of a second surgery in case of a wrong-level surgery.

The volume and the dosage of the injected mixture were chosen based on the authors' experience. The risk of using a large volume is that it could spread along multiple vertebrae and would therefore not indicate precisely the target IVD. Indeed, most cases in which the contrast medium extended beyond the mid body of the vertebrae adjacent to the injected IVD were injected with the larger volume (0.3 mL). It should however be noted that the marking by the methylene blue of the IVD and of the ventral soft tissues did not hinder the recognition accuracy of the anatomical landmarks used by the surgeon during the surgical approach.

On the postinjection x-rays, the contrast medium was clearly visible in at least 1 projection in all but 1 dog. This dog had a BCS of 7/9, with multiple soft tissue folds associated with marked wet-hair artifacts, which could explain the uncertainty. For the other cases, one could wonder if a single postinjection laterolateral view could be sufficient. However, the authors would advise performing both orthogonal views to increase the chances of clear visualization. The use of a CT scan, used as the gold standard in this study, could also be proposed as the imaging modality to confirm the site of injection, leaving no doubt and enabling better characterization of the mixture distribution.

During surgical dissection, the methylene blue was unclearly visible in 1/20 (5.0%) dogs. This case differed from the others in the fact that the vast majority of the mixture was injected within the central part of the IVD, with only a very small volume marking the ventral soft tissues located paramedianly (between the longus colli and intertransversarii ventralis cervicis muscles), which were therefore not visualized during surgical dissection. During surgical dissection, the dye was therefore only visible once the IVD had been identified and the ventral portion of the annulus fibrosus had been excised. This is suboptimal as at this stage of the surgery the soft tissues would have been traumatized and the IVD partially excised without the preventive assistance of the dye to help confirm the correct location of the target IVD. This underlines the importance of marking the ventral soft tissues, most importantly those located as close as possible to the midline (median soft tissues in this study), and notably the longus colli muscle, so that the methylene blue can be visualized by the surgeon when arriving at the level of this muscle during the surgical approach.

The injection was estimated feasible in all studied IVDs (C2-C3 to C6-C7) in all but 1 dog. In the latter case, the most caudal feasibly injectable IVD was C5-C6, and the dog was large (41.0 kg) and had a BCS of 9/9. This was consistent with the rest of the results that showed that the dogs' median weights were significantly different between most caudal IVD spaces feasibly injectable (the median weight was significantly lower for C7-T1 than for C6-C7). The elements inhibiting a more caudal injection during the US-guided procedure, such as the sternum, are also met intraoperatively, based on one of the coauthor's experience, increasing the risks of wrong-level surgery and further justifying the use of the mixture injection as guidance. Also, if the target site is not injectable, it could be proposed to inject another location in proximity, which could be used as an additional landmark by the surgeon.

Finally, it should be noted that the described technique can also be used for intradiscal injection of other products and for obtaining disc samples in cases of suspected discospondylitis.[22,24,25]

This study had multiple limitations. The small size of the study population limited the statistical analysis; however, the main aim of the study was to demonstrate the feasibility of the technique. There is a confirmation bias considering the fact that a single operator was used for the US-guided injection and the analysis of the x-rays and CT scan images. We however tried to limit this bias by anonymizing and randomizing the x-rays, therefore blinding the main operator, and by blinding the surgeon in regards to the outcome of the US-guided injection. Multiple elements of the study were based on subjective evaluations. The cadaveric design of the study meant that some anatomical structures, such as the vascular system, could have been missed during the US, with the use of Doppler rendered useless. However, the at-risk regional structures, including the main regional vasculature, were inspected during the surgical dissection by the surgeon, and no damage was identified. A future study in a clinical setting is therefore needed to assess the method on live patients, although there would still be a risk of venipuncture if the jugular vein, for instance, is compressed due to probe pressure.[16]

Based on the current findings in a population of cadavers, ultrasonographical cervical IVD identification and US-guided injection of IVD and ventral soft tissues are feasible and helpful for intraoperative cervical IVD identification. These results should be confirmed in a clinical setting.

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Supplementary Materials

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