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An anatomical training model for cerebrospinal liquid collection and myelography in embalmed dog cadavers*

Modelo anatômico para treinamento de colheita de líquido cerebroespinhal e mielografia em cadáveres de cães quimicamente preparados

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Abstract

In research and academic activities, guidelines are essential and imperative especially on the use of animals. Alternative methods that do not bring academic or scientific harm should also be sought. This study aimed to develop a training model for the collection of cerebrospinal fluid (CSF) and myelography in the cervical and lumbar regions in cadavers of embalmed dogs, using an alcoholic solution and curing salts for fixation and conservation. The dogs were divided into 4 grups of 8 animal each and stored between 2ºC and 6ºC, for 30, 60, 90, or 120 days. Durotomy was performed to implant two urethral catheters (one in the cranial direction and another in the caudal direction to the spinal cord access site), in the subduraracnoid space. This space was fixed via manual infusion of saline solution with a 20-mL syringe to simulate the presence of the CSF and the positive pressure, while the puncture was made. Four cadavers of each group were randomly selected for the CSF puncture from the atlantooccipital joint and in the lumbar region between L5 and L6, respectively, and four were used for CSF puncture training, in which radiographic contrast (myelography) was injected in the same locations. This model was cost-effective, did not utilize toxic products, and can preserve cadavers for up to 120 days. In this novel anatomical model, a maximum of 15 students can be trained on CSF puncture, allowing cervical and lumbar myelography and at least 30 perforations per cadaver.

Keywords: conservation, durotomy, fixation, imaging exams, perforation.

Resumo

É essencial e imperioso ter critério quanto ao uso de animais em pesquisa e atividades de ensino e, consequentemente, buscar métodos alternativos que não causem prejuízo acadêmico ou científico. Para que não ocorra deterioração dos tecidos, a fixação e conservação de peças anatômicas e cadáveres devem ser realizadas. Objetivou-se, com este estudo, desenvolver um modelo anatômico para treinamento de colheita de líquido cerebroespinhal (LCE) e mielografia, nas regiões cervical e lombar. Os cães foram divididos em quatro grupos contendo oito animais cada e armazenados entre 2ºC e 6ºC, por 30, 60, 90 ou 120 dias. Foi realizada durotomia para implantação de duas sondas uretrais, no espaço subaracnóide. A infusão manual de solução fisiológica com seringa de 20 mL foi utilizada para simular a presença do LCE e a pressão positiva, enquanto era feita a punção. Quatro cadáveres de cada grupo foram selecionados para a punção de LCE na articulação atlantooccipital e na região lombar entre L5 e L6, e quatro foram utilizados para o treinamento da punção de LCE e injeção de contraste radiográfico (mielografia). A técnica anatômica empregada possibilitou o desenvolvimento de um modelo visando ao ensino e pesquisa da radiologia em cadáveres de cães quimicamente preparados, a custo baixo e sem utilização de produtos tóxicos, mantidos sob refrigeração por 120 dias. Com isso, um máximo de 15 alunos podem ser treinados em punção do LCR, permitindo mielografia cervical e lombar com 30 perfurações por cadáver.

Palavras-chave: conservação, durotomia, exame de imagem, fixação, perfuração.

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Introduction

The use of toxic substances in anatomy laboratories have been increasingly controlled in Brazil and several other countries in consideration of occupational and environmental health. As many of these agents are used in the fixation and preservation of cadavers, residues that are harmful to the environment accumulate, and these contaminate the soil and water reserves. Thus, guidelines should be followed for the use of animals in research and teaching activities, and alternative methods that do not bring academic or scientific harm should be sought out (OLIVEIRA et al., 2013). The Law n. 11,794, called the Arouca Law, regulates the use of animals in research and teaching activities and establishes that the Ethics Committees in the Use of Animals (CEUA) should monitor teaching and research activities in universities, assist professionals in the biomedical area, and register the institution in the National Council for the Control of Animal Experimentation (BRASIL, 2008). There has been a growing demand for the creation of new techniques aimed at teaching animals.

The lack of practical training for collection of CSF during graduation becomes a limiting factor, as many fresh graduates of veterinary medicine consider themselves unfit and insecure to perform most clinical procedures, such as the collection of CSF and myelography (CAMPOS et al., 2016).

This study aimed to develop a training model for the collection of CSF and myelography in embalmed dog cadavers.

Materials and methods

Thirty-two cadavers of adult dogs were used in this study. These dogs died from causes that did not involve evident morphological alterations, such as tumor masses or bone fractures. The animals were frozen in a freezer at −18°C immediately after death.

Animals weighing between 5 and 15 kg and with a body condition score of 4 (considered "ideal"; score range, 1–9) were selected, based on a scaling system by Laflamme (LAFLAMME, 1997).

All animals were thawed in a refrigerator (2°C–6°C) and later weighed on a digital scale. All were shaved with stainless steel razor blades and injected with 150 mL/kg pure ethyl alcohol solution with 5% glycerin followed by 120 mL/kg of a solution containing 20% sodium chloride, 1% sodium nitrite, and 1% sodium nitrate via the common carotid artery. Then, the animals were placed in transparent plastic bags and kept refrigerated at the same temperature for preservation.

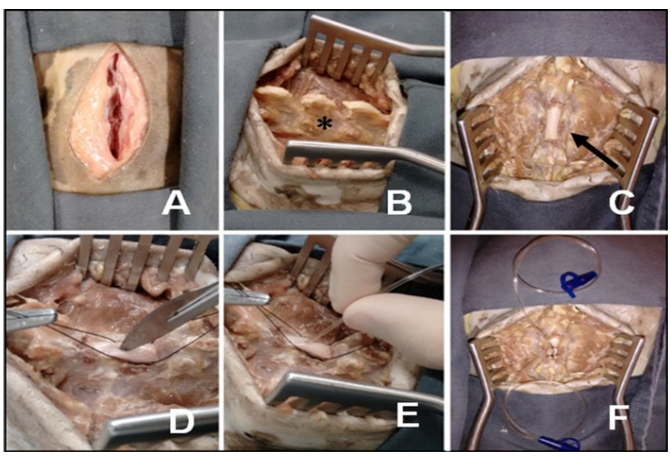
The 32 cadavers were randomly divided into four groups of eight animals each, based on the period (days) of preservation under refrigeration (Table 1).

Table 1: Average weight and standard deviation of dog cadavers and preservation time per group (G1: 30 days of preservation; G2: 60 days of preservation; G3: 90 days of preservation; G4: 120 days of preservation)

Group	Number of cadavers	Weight (kg)	Conservation time (Days)
G1	8	8.55±2.97	30
G2	8	9.01±3.12	60
G3	8	8.02±3.07	90
G4	8	7.63±3.55	120

In the last week of preservation in each group, a surgical procedure was performed on the cadavers for further practical training. A surgical access was made in the thoracolumbar region of the spine, via a modified dorsal laminectomy between L2 and L4 (Figure 1) (FOSSUM, 2008). From the exposed spinal cord, durotomy was performed (TUDURY and POTIER, 2009) to implant two urethral catheters n° 4 or 6 (one in the cranial direction and another in the caudal direction to the spinal cord access site), depending on the size of the cadaver, in the subaracnoid space. This space was fixed via manual infusion of saline solution using a 20-mL syringe to simulate, the presence of the CSF and the positive pressure, while the puncture was made (Figure 2) and the liquid came out in excellent flow, like alive animals. The catheters were duly identified with tape labels based on their directions (cranial or caudal). A ligature with a nylon 2-0 thread was used, involving the spinal cord and the catheters. Synthesis was conducted with simple continuous suture pattern to reduce the subcutaneous space.

Figure 1: Surgical preparation of a embalmed dog cadaver for CSF collection and myelography. A: Surgical access in the thoracolumbar region. B: Visualization of vertebral spinous processes (*); C: Exposed dura mater after removal of the spinous processes (arrow). D: Durotomy for implantation of urethral catheters. E: Subaracnoid insertion of an urethral catheter to restore positive pressure after application of saline solution. F: urethral catheters in cranial and caudal directions inside the subaracnoid space.



In each group, four cadavers were randomly selected for the CSF puncture from the atlantooccipital joint and in the lumbar region between L5 and L6, respectively, and four for CSF puncture training, including injection of radiographic contrast (myelography) in the same locations.

The space between the occipital and atlas (GAMA et al., 2009) and the space between L5 and L6 (FELICIANO et al., 2015) were selected for the puncture of the simulated CSF and injection of contrast in the myelography. Myelography was performed only once in the Diagnostic Imaging Sector of the Veterinary Hospital "Governador Laudo Natel," School of Agrarian and Veterinary Sciences FCAV, UNESP Campus de Jaboticabal - SP because after the injection of the radiographic contrast solution, the cadaver could no longer be used. The procedures were performed with a 0.80 × 25 mm^2 or 0.80 × 30 mm^2 hypodermic needle or similar, according to the size of

the animal. Iohexol contrast was injected at a dose of 0.30 mL/kg into the atlantooccipital joint and 0.45–0.50 mL/kg into the lumbar region (FELICIANO et al., 2015). A qualitative analysis of the cadavers included the morphological parameters such as flexibility, malleability, softness, color, and odor, to check whether these were preserved as close to the live animals as possible.

Figure 2: Embalmed dog for CSF collection and myelography. The manual infusion of saline solution simulates the CSF while the student performs the puncture in the lumbar region between L5 and L6.

Anova and Kruskalwalis´ tests were performed to check diferences among groups as for the number of perforations on atlantooccipital and L5-L6 joints.

Results

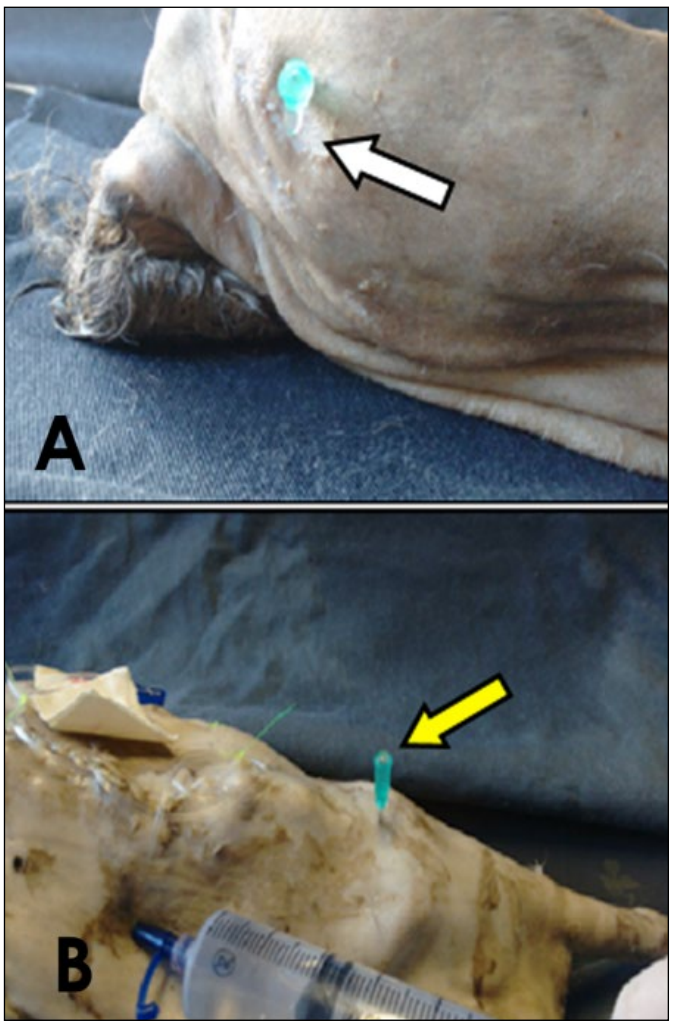
The use of this anatomical technique using the alcoholic solution and curing salts was efficient for the fixation of the dogs' cadavers during the 120 days of preservation. During storage in refrigerators (2**°C**–6°C), the cadavers did not release fat and transudate in the plastic bags, and handling was not difficult, which facilitated the surgical preparation of the modified dorsal laminectomy and implantation of urethral catheters in the subarachnoid space for the CSF puncture and myelography techniques.

Moreover, no stiffening of the cadavers was observed. The morphological characteristics such as flexibility, malleability, and maintenance of the color of the muscles, skin, and organs were preserved. The cadavers did not present a fetid odor or evidence of putrefaction at any periods of evaluation.

The chemical preparation of the dogs was not different between groups (after 30, 60, 90, and 120 days).

In all groups, each cadaver was punctured on the atlantooccipital joint and between L5 and L6. As soon as the subarachnoid space was reached by the needle, the simulated CSF (saline solution) leaked through the needle while maintaining positive pressure using the syringe plunger (Figure 3).

Figure 3: A: Fluid overflow through the needle (white arrow) in CSF collection between the occipital and the atlas in an embalmed dog; In B, overflow of fluid through the needle (yellow arrow) inserted in the lumbar region.



In both spaces, between the atlantooccipital joint and in the lumbar region, a great number of perforations were made in the chemically prepared dogs, which is essential for teaching and training for the CSF puncture and myelography (Tables 2 and 3).

Table 2: Mean and standard deviation (SD) of the number of perforations in each cadaver for CSF puncture in the atlantooccipital joint in embalmed dogs. G1: 30 days of preservation; G2: 60 days of preservation; G3: 90 days of preservation; G4: 120 days of preservation

	G1	G2	G3	G4
MEAN	33.50	29.75	30.75	28.25
SD	4.04	7.09	2.63	5.12

Table 3: Mean and standard deviation (SD) of the number of perforations in embalmed dog cadavers for CSF puncture between L5 and L6. G1: 30 days of preservation; G2: 60 days of preservation; G3: 90 days of preservation; G4: 120 days of preservation

	G1	G2	G3	G4
MEAN	31.50	24.25	24.75	25.50
SD	2.38	5.19	1.71	1.73

After performing the CSF technique in the atlantooccipital joint and the space between L5 and L6, in the lumbar region, the contrast was injected to perform myelography. Radiographic examinations were performed sequentially, confirming the effectiveness of myelography in both regions after visualizing good evolution and sharpness of the dorsal and ventral contrast column, as verified by the increase in radiopacity that delimited the spinal cord (Figure 4 and 5).

Figure 4: A: Simple lateral cervical radiographic image and the needle in CSF puncture (arrow) in a embalmed dog cadaver; B: Myelography and the evidence of the contrast column delimiting the spinal cord in the cervical region (arrow).

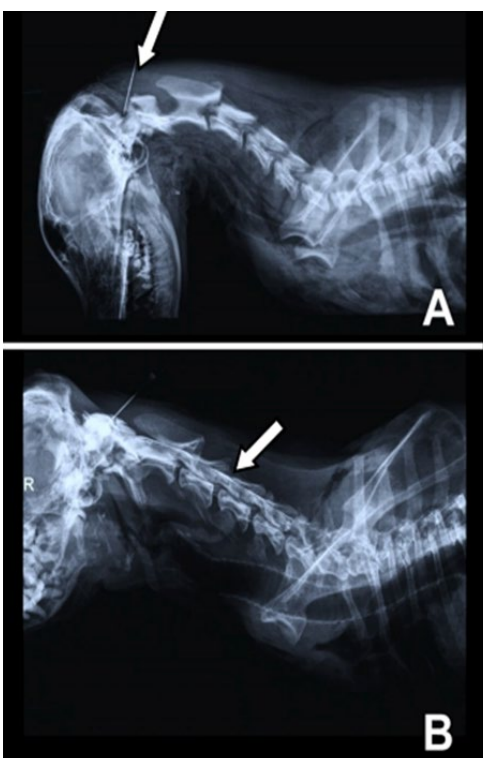
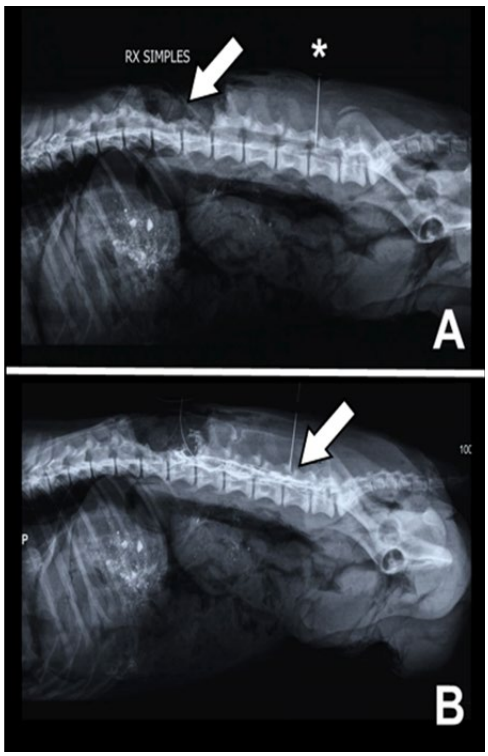


Figure 5: A: Simple lateral radiographic image and the urethral catheter at L2 and L3 (arrow) and the needle between L5 and L6(*) in a embalmed dog cadaver; B: Myelography and the contrast column delimiting the spinal cord in the lumbar region (arrow)



There was no statystical difference among groups as for the number of perforations on atlantooccipital and L5-L6 joints by Anova and Kruskalwalis´ tests.

Discussion

Traditional procedures for preparing cadavers using formaldehyde are limited for teaching and surgical training due to changes in tissue color and resistance. On the other hand, artificial anatomical models can be used several times, unlike fresh cadavers (GROSCURTH et al., 2001). However, in this study, the formaldehyde-free embalmed cadavers were kept for one academic semester (120 days), which provided and enabled training such as the collection of CSF and the performance of myelography, and maintained softness and tissue malleability.

The ethyl alcohol solution is an efficient fixative, in human cadavers for up to 1 year (GOYRI-O'NEILL et al., 2013). During fixation, ethyl alcohol is followed by an aqueous solution of 30% sodium chloride to preserve cadavers of dogs and cats for up to 4 months, to be used in surgical training involving the skin and jejunum (ROCHA et al., 2018; DEL PONTI et al., 2021; FERREIRA et al., 2021, QUEIROZ et al., 2022a; QUEIROZ et al., 2022b), arteries (CERQUEIRA et al., 2017), and veins (PELOGIA et al., 2018).

The use of salt solution in anatomical preparation can preserve cadavers for long periods, maintain color and softness, and avoid contamination and unpleasant odors, in addition to being low cost and without environmental and health risks (JANCZYK

et al., 2010; WELDERMANN and GERICS, 2016). In this study, a solution comprising chloride, nitrite, and, sodium nitrate preserved the color, malleability, and texture of the tissues, prevented macroscopic contamination, was prepared at a low cost, and did not have risks to health and the environment. Thus, the proposed practices were similar to those for studies investigating bone marrow quality in chemically prepared dogs that were fixed and preserved with the same solution (ROCHA et al., 2022).

The maintenance of chemically prepared cadavers under refrigeration for up to 4 months did not reveal signs of contamination and putrefaction, guaranteed by the reduction of the development of microorganisms, as in the preservation of meat products (ROSSET, 1994) and in studies that kept embalmed cadavers of dogs and cats under refrigeration for up to 4 months (DEL PONTI et al., 2021; FERREIRA et al., 2021; QUEIROZ et al., 2022a; QUEIROZ et al., 2022b).

The alcoholic solution and curing salts avoided contaminated effluents, commonly observed when toxic preservatives are used (WHO, 1991) or odors that are harmful to health as those released by formaldehyde (CURY et al., 2013). In addition, appropriate waste management of these agents are assocaited with a high financial and environmental cost, thus the need to search for alternatives that do not present risks (JANCZYK et al., 2010).

Tissue alterations in animal cadavers have already been observed when the fixation agent is formaldehyde, which causes stiffening in chicken breasts fixed for 45 days (7 times longer) or even for a year (4–5 times greater) (GUASTALLI et al., 2012a; GUASTALLI et al., 2012b). Ethyl alcohol used as a fixative increases tissue stiffness, almost five times stiffer during the first 6 months or three times stiffer after 1 year of immersion in this agent (NUNES et al., 2011). In contrast, the findings of the present study demonstrated no change in tissue resistance during the 120 days of cadaver preservation, suggesting the technique's efficiency.

Practical skills and theoretical knowledge are required in health courses such as veterinary medicine. However, several factors such as the number of students, lack of adequate opportunities, ethical debates, and issues related to animal welfare have discouraged teachers and students, contributing to a deficiency in the clinical and surgical skills of the future professionals (CAPILÉ et al., 2015). Resident veterinarians and fresh graduates of veterinary medicine who complete a self-assessment questionnaire regarding acquired clinical skills (e.g., collection of CSF from the atlantooccipital joint and lumbar region) have considered themselves unfit (70.7% and 72.8%, respectively) to perform this technique (CAMPOS et al., 2016). Embalmed cadavers will be an alternative for training and learning skills for the CSF puncture technique in the atlantooccipital joint and lumbar region and for the myelography technique, as real conditions can be simulated. This model guarantees a high rate of repeatability per cadaver, as supported by the number of perforations in all evaluation time points of this study. It was possible to perform more than 24 lumbar or 28 atlantooccipital perforations in all groups, which were very uniform in terms of conservation over the 4 months evaluated and with no statistical difference between them.

The use of dog cadavers preserved with ethyl alcohol and sodium chloride solution, intended for surgical training, provided a good evaluation of the undergraduates of the Veterinary Medicine course, with 75.67% approval of the conservation technique, 81.08% approved the initial training on cadavers, followed by practice on live animals (ROCHA et al., 2019). Similar values were observed when the same anatomical technique was used in the preparation of cat cadavers, with the same purpose, in which 92% (ZERO et al., 2020) or 93% (SILVA et al., 2003) of the students were in favor of the use of chemically prepared cadavers in the teaching of the surgical technique and 90% opted for training with cadavers followed by practice on live animals (ZERO et al., 2020). In embalmed dogs, 100% of the students considered using cadavers to perform surgical techniques. All students preferred the initial training in embalmed cadavers, followed by surgery classes with live animals (DEL PONTI et al., 2021). Such data demonstrate the acceptability of veterinary medicine students for the use of chemically prepared cadavers for teaching and training clinical and surgical procedures, justifying the use of the training model in this study.

The embalmed cadavers for training were in accordance with the legislation of the Arouca Law (n.11.794) and involves alternative and ethical teaching methods. The practice of collecting CSF and myelography in the proposed models reduced costs and provided repeatability. In addition, as already observed in other universities, there is considerable receptivity from undergraduates and residents (KNIGHT, 2007). The development of alternative models to the use of live animals is a worldwide trend, and currently, they are used in several universities worldwide, such as Canada and the United States, for training undergraduates, thus preventing euthanasia of thousands of animals (BALCOMBE, 2000).

The anatomical technique preserved the cadavers for a long period, in a realistic way for teaching, as desired (BALTA et al., 2015). Moreover, new protocols are needed to help anatomists in the preparation of embalmed cadavers, with high-quality and beneficial tests of new radiographic equipment and minimally invasive surgery, thereby encouraging more scientific experiments.

Conclusions

The anatomical technique used for the fixation and conservation of dogs cadavers using the alcoholic solution and curing salts, as well as, the surgical preparation aiming to reestablish and simulate the cerebrospinal fluid, proved to be effective, simulating a real condition (live animal), with considerable durability, without unpleasant odor, without loss of the morphological characteristics, easy storage, and handling. It is also a great model that respects the bioethical standards of animals to be used for teaching.

The method allows the training of cervical and lumbar puncture for collection of CSF and myelography, simulating real conditions, with high repeatability.

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