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Comparative *ex vivo* morphometric tomographic study of lumbar spine between dog and rabbit

[*Estudo morfométrico tomográfico* ex vivo *de coluna lombar comparativo entre cão e coelho*]

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ABSTRACT

The study of spinal surgery in dogs has limitations for the standardization of the sample, due to the difficulty of obtaining animals of the same breed, sex, and age. Thus, the use of the rabbit as an animal model is suggested. Morphometric studies are important to assess the anatomical compatibility between the two species. Furthermore, the growing interest in the rabbit as a pet and the common occurrence of iatrogenic fractures in its caudal lumbar spine emphasize the need for these studies. Eight spinal segments (L1-S3) from adult dogs, between 3 and 5kg, and eight from New Zealand rabbits, between 3 and 5kg, adults, were submitted to cone beam computed tomographic examination. In the generated images, the length, height and width of the body and vertebral foramen were measured, in addition to the thickness of the cortical bone. The lumbar vertebrae of rabbits are longer than those of dogs, but they are smaller in width and height. The thickness of the cortical bone of the two species is similar. The morphometric differences found restrict the use of the rabbit as an animal model for the development of experimental surgeries in dogs.

Keywords: *Canis lupus familiaris*, cone beam computed tomography, cortical bone, *Oryctolagus cuniculus*, spine

RESUMO

Os estudos de cirurgias da coluna vertebral de cães apresentam limitações para a padronização da amostra, devido à dificuldade de se conseguir animais de mesma raça, sexo e idade. Dessa forma, sugere-se o uso do coelho como modelo animal. Estudos morfométricos são importantes para avaliar a compatibilidade anatômica entre as duas espécies. Além disso, o crescente interesse pelo coelho como animal de estimação e a ocorrência comum de fraturas iatrogênicas na coluna lombar caudal enfatizam a necessidade desses estudos. Oito segmentos de coluna vertebral (L1-S3) de cães adultos, entre 3 e 5kg, e oito de coelhos adultos, da raça Nova Zelândia, entre 3 e 5kg, foram submetidos a exame tomográfico computadorizado de feixe cônico. Nas imagens geradas, foram mensurados o comprimento, a altura e a largura do corpo e do forame vertebral, além da espessura do osso cortical. Pode-se observar que as vértebras lombares dos coelhos são mais compridas do que as dos cães, porém apresentam largura e altura menores. A espessura do osso cortical das duas espécies é semelhante. As diferenças morfométricas encontradas restringem o uso do coelho como modelo animal para o desenvolvimento de cirurgias experimentais de cães.

Palavras-chave: Canis lupus familiaris, *coluna vertebral*, Oryctolagus cuniculus, *osso cortical*, *tomógrafo computadorizado de feixe cônico*

INTRODUCTION

The development of new surgical techniques for the treatment of spinal disorders in dogs is a challenge. Several in vitro biomechanical studies of isolated vertebral segments have been performed in dogs to quantify the effects of surgical changes on the spine (Hofstetter *et al.*, 2009; Bösch *et al.*, 2017; Selz *et al.*, 2020). However, in intervertebral arthrodesis, the *in*

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vivo influence of the stabilizing epaxial musculature, muscular fasciae and abdominal musculature is not evaluated (Selz *et al*., 2020), as is the absence of fatigue tests for the fixation method, the level of stiffness required for long-term stability and fusion remain uncertain, and further *in vivo* studies are needed to recommend the routine use of the method (Schöllhorn *et al*., 2013).

In experimental surgery, there is an attempt to use less medium-sized animals, such as dogs and pigs, and progressively to use small-sized animals, such as rats, mice, guinea pigs and rabbits, due to the lower cost, ease of obtaining, handling, and maintenance (Hossne, 2003). Thus, the choice of the rabbit as an animal model for the development of experimental surgeries of the spinal column of dogs, is because they are the largest mammals, among the available laboratory animals, and because they have less social appeal than dogs (Fagundes and Taha, 2004). It is necessary to validate the extrapolation from one species to the other, requiring a critical analysis of the limitations inherent to anatomical differences.

In addition, the demand for rabbits as companion animals grows annually, making them the third most common mammalian pet in the United States and United Kingdom (The pet…, 2018; Crowell-Davis, 2021). Fractures and dislocations of the lumbar spine are common in these animals due to their fragile skeleton and well-developed musculature in the pelvic limbs. Sudden and explosive muscle activity causes hyperextension of the lumbosacral joint, which can generate spinal cord trauma, most often involving the seventh lumbar vertebra (Greenaway *et al.*, 2001; McCullough *et al*., 2012). Classically, this type of injury is the result of inadequate restraint (Greenaway *et al*., 2001; Fisher and Carpenter, 2012). Affected animals may show a range of clinical signs and varying degrees of neural impairment (Fisher and Carpenter, 2012; McCullough *et al*., 2012). Despite the relatively common nature of the lesion, the veterinary literature lacks information on the clinical success of surgical decompression and vertebral stabilization methods in rabbits (McCullough *et al*., 2012; Delamaide *et al*., 2014; Moran *et al.*, 2017). This can be partially attributed to the fragile nature of the spine, the expense associated with spinal surgery, the infrequency of advanced diagnostic testing in rabbits, and the need for properly trained veterinarians to perform the surgery (Delamaide *et al.*, 2014).

The use of rabbits in research, as well as their growing popularity as pets, emphasizes the need for detailed information about the anatomy of rabbit vertebrae. Veterinary orthopedic surgeons are familiar with the surgical anatomy of the dog and would possibly benefit from understanding rabbit anatomy in a comparative way.

The present tomographic morphometric study aimed to compare the anatomical structure of the lumbar vertebrae and the thickness of the cortical bone between dogs and rabbits. This information is important to assess the limitations of using the rabbit as an animal model for surgical techniques on the lumbar spine of dogs, and to offer support for planning the surgical treatment of conditions in this region in rabbits.

MATERIAL AND METHODS

Sixteen spinal column segments (L1-S3) were used, eight from dog cadavers (*Canis lupus familiaris*) and eight from rabbits (*Oryctolagus cuniculus*). The animals had between 3 and 5 kg of body mass, were young adults and there was no predilection for sex. The dogs came from veterinary clinics and hospitals in Curitiba-PR, being ethical corpses, that is, they died due to conditions unrelated to the study. The rabbits were of the New Zealand breed and came from another scientific experiment, with authorization from the ethics committee under number 056/2017. For both species, the inclusion criterion in the study was the absence of a history of neurological signs of lumbar spinal cord injury, bone or nutritional diseases, fractures, dislocations, vertebral neoplasms, congenital diseases, or a history of lumbar spine surgery. Also, animals with anatomical abnormalities visible on digital radiographic examination in double exposure were not included. To produce the specimens, the epaxial musculature, the joints and the ligaments of the spinal segments were kept intact, being stored at -20°C, moistened with physiological solution and packed with PVC (polyvinyl chloride) film paper. Aiming at thawing for the CT scan, the day before the study, the specimens were transferred and stored in a refrigerator at +4°C.

The tomographic images were obtained using the Cone Beam Computed Tomography (CBCT), i-CAT® Next Generation model (*Imaging Sciences Internationa*l, Hatfield, PA, USA), belonging to the Laboratory of Teaching and Research in Imaging of the Federal University of Paraná (LABIM – UFPR). The FOV (*field of view*) and voxel size were set at 16 by 8 cm and 0.2 mm, respectively. Tube current and voltage and exposure time were preset at 5 mA, 120 kVp and 14.7 s, respectively. The specimens were placed on a fixed support with a 15 cm long by 15 cm wide base and positioned in the craniocaudal and dorsoventral directions. The images were acquired using the *Imaging Sciences International* software, developed by the same manufacturer as the CBCT, and the reconstructions were later analyzed using the free software RadiAnt DICOM Viewer BETA 4.9.15.

To compare anatomical aspects between dogs and rabbits, the images were reconstructed using the Multiplanar Reconstruction (MPR) tool. The steering axes were positioned so that they crossed at the central point of the vertebral body of the vertebra under analysis in the sagittal, transverse, and longitudinal planes at the same time (Fig.1).

Figure 1. Tomographic sections of the fourth lumbar vertebra of a dog, Lhasa Apso, male, four years old, with direction axes positioned at the central point of the vertebral body, using the Multiplanar Reconstruction tool. 1 – Sagittal plane with the X axis positioned parallel to the back of the vertebral body; 2 – Longitudinal plane; 3 – Transverse plane with Y axis over the spinous process of L4.

With the vertebra properly positioned according to the reference axes, the length, width and height of the body and vertebral foramen were measured. In the sagittal plane, the length (Fig. 2.1b) and height (Fig. 2.1a) of the vertebral foramen were measured, in addition to the height of the vertebral body (Fig. 2.1c). In the longitudinal plane, the width (Fig. 2.2d) and length (Fig. 2.2e) of the vertebral body were measured. The width of the vertebral foramen was measured in the transverse plane (Fig. 2.3f). In addition, cortical bone thickness was measured in the ventral part of the vertebral body, also in the transverse plane (Fig. 2.3e).

To assess the degree of reliability of the morphometric assessment, all measurements were repeated 3 times with an interval of at least 1 week between measurements, always performed by the same evaluator.

Data were tabulated in Excel and statistics were performed using *Graphpad prism v5*. Descriptive statistics of the parameters studied were calculated, such as mean, standard deviation, median, minimum, and maximum value. To choose the statistical test, the Grubbs test was performed to rule out outliers and the D'Agostino-Pearson normality test. The data showed normal distribution and as they are unpaired samples, the T test was used for statistical analysis and comparison between groups.

Figure 2. Tomographic sections of the fourth lumbar vertebra of a dog, Lhasa Apso, male, four years old, using the Multiplanar Reconstruction tool to measure (a) height and (b) length of the vertebral foramen; (c) height of the vertebral body in the sagittal plane (1); (d) width and (e) length of the vertebral body in the longitudinal plane (2); (f) width of the vertebral foramen and (g) thickness of the cortical bone in the transverse plane (3).

RESULTS

Both groups were uniform in relation to the animals' body mass, with no statistical difference ($p<0.05$), and the average weight of the dogs was 4.188 kg (Standard Error ± 0.3969, n=8) while that of rabbits was 3.288 kg (Standard Error ± 0.3054, n=8).

The length of the vertebral body (L1-L7) of the rabbit is 23% greater than that of the dog ($p<0.0001$). While the height and width are significantly smaller than that of the dog's lumbar vertebra, being 36% and 96% smaller, on average, respectively ($p<0.0001$; Table 1).

Table 1. Mean and Standard Error of the average measurements of the length, height, and width of the vertebral body of lumbar vertebrae compared between dogs (n=8) and rabbits (n=8), in millimeters

Vertebra	Vertebral Body					
	Length (mm)		Height (mm)		Width (mm)	
	dog	rabbit	dog	rabbit	dog	rabbit
L1	13.12±0.37*	17.94±0.49*	5.71±0.22*	3.62±0.13*	10.46±0.29*	4.56±0.11*
L2	13.69±0.37*	18.83±0.38*	5.99±0.22*	3.93±0.15*	10.66±0.30*	4.71±0.12*
L3	14.41±0.37*	19.25±0.37*	5.96±0.26*	3.64±0.08*	11.44±0.42*	4.82±0.14*
L4	14.77±0.38*	19.30±0.34*	6.01±0.29*	3.36±0.09*	10.77±0.39*	5.52±0.12*
L5	15.16±0.41*	19.02±0.35*	5.61±0.27*	3.58±0.13*	10.31±0.31*	5.85±0.14*
L6	14.53±0.38*	18.36±0.32*	5.76±0.29*	3.63±0.12*	11.42±0.44*	6.72±0.13*
L7	12.41±0.33*	16.03±0.30*	6.50±0.31*	4.54±0.14*	13.86±0.58*	8.03±0.19*

*statistical difference, considering p<0,0001

Regarding the rabbit vertebral foramen, the length is 23% longer, while the height and width are 33% and 26% shorter when compared to the dog's lumbar vertebra (p<0.0001; Tab. 2).

There was no statistical difference between the cortical bone thickness of the ventral part of the vertebral body in the lumbar region between dogs and rabbits (Table 3).

Table 2. Mean and Standard Error of measurements of the length, height, and width of the vertebral foramen of lumbar vertebrae compared between dogs (n=8) and rabbits (n=8), in millimeters

Vertebra	Vertebral Foramen					
	Length (mm)		Height (mm)		Width (mm)	
	Dog	Rabbit	Dog	Rabbit	Dog	Rabbit
L1	11.43±0.38*	15.15±0.37*	5.53±0.18*	3.65±0.12*	6.83±0.19*	4.89±0.18*
L2	11.93±0.34*	15.79±0.32*	5.78±0.17*	3.73±0.12*	7.22±0.19*	5.07±0.14*
L3	12.37±0.39*	16.29±0.27*	6.18±0.17*	3.45±0.09*	7.89±0.20*	5.17±0.11*
L4	12.74±0.35*	16.07±0.26*	6.37±0.19*	3.64±0.08*	8.72±0.20*	5.38±0.08*
L5	12.93±0.36*	15.90±0.27*	5.72±0.20*	4.15±0.10*	8.96±0.27*	6.29±0.15*
L6	11.81±0.30*	15.29±0.29*	5.07±0.16*	4.14±0.09*	8.88±0.24*	7.03±0.18*
L7	9.26±0.25*	12.76±0.32*	4.40±0.14*	3.39±0.11*	9.66±0.19*	7.94±0.22*

*statistical difference, considering p<0,0001

Table 3. Mean and Standard Error of the cortical bone thickness of lumbar vertebrae compared between dogs (n=8) and rabbits (n=8), in millimeters

Specie	Cortical bone thickness ± Standard Error (mm)						
	L1	L2	L3	L4	L5	L6	L7
Dog	0.65±0.03	0.70±0.03	0.70±0.03	0.71±0.02	0.75±0.03	0.73±0.02	0.73±0.02
Rabbit	0.62±0.02	0.63±0.02	0.64±0.02	0.68±0.02	0.68±0.02	0.69±0.02	0.72±0.02

DISCUSSION

The choice of CBCT was because the specimens are small, and the device allows cuts of up to 0.2 mm, whereas traditional CT scanners have thicker cuts, ranging from 0.5 to 20 mm (Garib *et al.*, 2007). This type of tomograph is relatively small, has a lower cost and was originally developed for the oral and maxillofacial region. The main advantage is the reproduction of the three-dimensional image of mineralized tissues, with minimal distortion (Scarfe *et al.*, 2006), which is interesting for morphometric studies. Several studies have already validated the accuracy of measurements performed on these images and the reliability of this measurement method, considered the gold standard (Scarfe *et al.*, 2006; Moshiri *et al.*, 2007; Na *et al.*, 2019).

In addition to being highly accurate, the CBCT image distinguishes between enamel, dentin, pulp cavity and alveolar cortical bone (Hashimoto *et al.*, 2003), which allowed the assessment of cortical bone thickness in this study. Although the cortical bone of rabbits is thinner than that of carnivores in clinical books on exotic animals and that this characteristic makes it difficult to place bone plates (Fisher and Carpenter, 2012; Pessoa, 2014), there was no statistical difference in this regard, when compared to the vertebrae of the lumbar region of the dog and rabbit group. Since the cortical bone thickness of the rabbit is similar to that of the dog, further studies should be carried out to verify other parameters that may justify this greater difficulty in using bone plates in the species, such as comparative studies of bone density between the two species. This characteristic cannot be evaluated in this study, because CBCT does not allow measurement, unlike traditional tomography.

This comparative tomographic morphometric study allowed us to observe that the vertebral body of the rabbit is longer, but the central part has a width and height significantly smaller than those of the dog, reaching a reduction of 96% in width, that is, the vertebral length is larger, but the central region is very narrow. This factor may help explain the greater vertebral fragility observed in rabbits and the common occurrence of iatrogenic fractures (Fisher and Carpenter, 2012; McCullough *et al*., 2012)

Moran *et al.* (2017) performed an *ex vivo* tomographic study of the thoracolumbar spine of rabbits, which aimed to evaluate the anatomy and define safe corridors for implant placement, in addition to placing pins and testing these previously established corridors. The authors note that although safe corridors exist, there is a high risk of insertion errors due to the small size of the rabbit's vertebrae. While the entire vertebral length can be utilized for implant placement in dogs, the limited vertebral anatomy of rabbits does not allow for this use. The authors report that the largest bone volume is contained in the narrow cranial and caudal epiphyses, while in the center of the vertebral body, the bone thins to the point where the thickness is inadequate for implant placement. The difficulty reported by the authors was proven and better defined in the present study. Surgical planning for the treatment of fractures and dislocations of the vertebral column in adult New Zealand rabbits, with approximately 3.2 kg of body mass, should consider that the mean length of the vertebral body of rabbits is 18.39 mm and the height and width, in the central region, are approximately 3.75 mm and 5.75 mm, respectively. These difficulties can be overcome with the use of surgical guides generated in 3D printers from CT scans.

The vertebral foramen of the rabbit showed the same pattern as the vertebral body, being longer, but with a width and height smaller than those of the dog. As the vertebral foramen of the rabbit is narrower, this fact suggests greater ease in compressive spinal cord injuries in this species. Rabbits have a minimal cauda equina and the spinal cord ends at the level of the second sacral vertebra (Greenaway *et al.*, 2001), while in the dog, it ends at the sixth or seventh lumbar vertebra (Sisson, 2013), which justifies the greater degree of neural impairment in fractures of the seventh lumbar vertebra in rabbits.

Due to the anatomical differences observed in this morphometric study, we cannot consider the rabbit as a suitable animal model for the development of experimental surgeries of the lumbar spine of the dog, since implants dedicated to the vertebral anatomy of the rabbit do not apply to the dog. Furthermore, due to the delicate anatomy of the rabbit vertebra, there is a greater risk of trans and postoperative complications in this species than in the dog, therefore, if a technique tried in the rabbit is considered unsuccessful, extrapolation of this information to the dog may be a misconception.

However, our study has some limitations. Firstly, only one breed of rabbit was examined and variations in vertebral morphology may occur between breeds. Secondly, the group of dogs was not uniform for presenting animals of different breeds, both chondrodystrophic and non-chondrodystrophic. Finally, a single observer performed all measurements, making it impossible to assess the interobserver correlation.

CONCLUSION

The lumbar vertebrae of the rabbit are longer, but they are smaller in width and height when compared to those of small dogs. The thickness of the vertebral cortical bone of dogs and rabbits is similar. The morphometric differences observed in the lumbar region of the spine limit the use of the rabbit as an animal model for the development of experimental surgery in dogs.

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