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## **RESEARCH ARTICLE**

### Calculation of Intracranial Volume in Akkaraman and Kangal Akkaraman Sheep by Stereology and Computed Tomography

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### Akkaraman ve Kangal Akkaraman Koyunlarında Stereoloji ve Bilgisayarlı Tomografi ile İntrakraniyal Hacmin Hesaplanması

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#### Öz

Amaç: Koyun ırklarının intrakraniyal hacminin bilinmesi ırk ayrımı ve klinik bilimler açısından önemlidir. Bu çalışmanın amacı Türkiye'deki koyun varlığının büyük bir çoğunluğunu oluşturan Akkaraman ve Kangal Akkaraman koyun ırklarının intrakraniyal hacimlerinin ve ölçüm yöntemlerinin karşılaştırılmasıdır.

Gereç ve Yöntem: Çalışmada sağlıklı erkek 6-8 aylık yaşta 10 adet Akkaraman ve 10 adet Kangal Akkaraman koyununa ait başlar kullanıldı. Koyun başları rostral-caudal yönde, kesit oryantasyonuna referans sağlamak için sert damağa dik, 0,6 mm dedektör kalınlığı ile aksiyel (transversal) planda görüntüleme yapıldı. Aksiyel görüntülerin rekonstrüksiyonu 0,65 mm kesit kalınlığı ile yapıldı. Koyun başlarının intrakraniyal hacimleri bilgisayarlı tomografi (BT) görüntüleri kullanılarak Cavalieri prensibi ile ölçüldü. Ek olarak Slicer 5.3 programı ile intrakraniyal alanın üç boyutlu modelleri oluşturuldu ve program araçları ile hacmi hesaplandı. Hacim ölçümü sonucu elde edilen veriler bağımsız gruplarda t testi, metotlar arasındaki karşılaştırma ise Bland-Altman testi ile SPSS 26.0 paket programında analiz edildi.

**Bulgular:** Akkaraman koyununun intrakraniyal hacminin ortalama değerlerinin Akkaraman koyunlarından istatistiksel olarak anlamlı ve büyük olduğu bulundu (P<0.01). Stereolojik yöntem ve BT modelleri ile hesaplanan intrakraniyal hacimlerin istatistiksel olarak karşılaştırılması sonucunda iki metot arasında fark olmadığı tespit edildi. (P>0.05).

Öneri: İntrakraniyal hacmin doğru bir şekilde hesaplanması, beyin ve sinir sistemi hastalıklarının teşhis ve tedavisinde büyük bir öneme sahiptir. Bu çalışmanın sonuçları, Akkaraman ve Kangal Akkaraman koyunlarında intrakraniyal hacmin belirlenmesinin hastalıkların tanısında klinik bilimlere önemli katkılar sağlayabileceğini göstermektedir. Aynı zamanda veteriner hekimler ve araştırmacılar için hastalıkların daha iyi anlaşılması ve etkili tedavi yöntemlerinin geliştirilmesinde önemli bir rehberlik sağlayabilir. Bu nedenle, çalışmanın sonuçları veteriner tıp ve ilgili disiplinlerde yapılan araştırmaları yönlendirebilir ve klinik uygulamalar için önemli bir temel oluşturabilir.

#### Abstract

**Aim:** Knowing the intracranial volume of sheep breeds is important for breed segregation and clinical sciences. The aim of this study is to compare the intracranial volumes and measurement methods of Akkaraman and Kangal Akkaraman sheep breeds, which constitute the majority of sheep in Turkey.

**Materials and Methods:** In the study, the heads of 10 healthy male Akkaraman and 10 Kangal Akkaraman sheep aged 6-8 months were used. The sheep heads were imaged in the rostral-caudal direction, perpendicular to the hard palate to provide reference for the section orientation, in the axial (transversal) plane with a detector thickness of 0.6 mm. Reconstruction of the axial images was done with a slice thickness of 0.65 mm. Intracranial volumes of the sheep heads were measured using computerized tomography (CT) images with the Cavalieri principle. In addition, three-dimensional models of the intracranial area were created with the Slicer 5.3 program and the volume was calculated with the program tools. The data obtained from volume measurement were analyzed with the t-test in independent groups, and comparison between methods was analyzed with the Bland-Altman analysis in the SPSS 26.0 package program.

**Results:** The mean values of intracranial volume of Akkaraman sheep were found to be statistically significant and greater than Akkaraman sheep (P<0.01). As a result of statistical comparison of intracranial volumes calculated with stereological method and CT models, no difference was found between the two methods (P>0.05).

**Conclusion:** Accurate calculation of intracranial volume is of great importance in the diagnosis and treatment of brain and nervous system diseases. The results of this study show that the determination of intracranial volume in Akkaraman and Kangal Akkaraman sheep can provide significant contributions to clinical sciences in the diagnosis of diseases. Therefore, it can also provide important guidance for veterinarians and researchers in better understanding diseases and developing effective treatment methods. Therefore, the results of the study can guide research in veterinary medicine and related disciplines and provide an important basis for clinical applications.

Anahtar kelimeler: 3B modelleme, Akkaraman koyunu, İntrakraniyal hacim, Kangal Akkaraman koyunu, Stereoloji. **Keywords:** 3D modeling, Akkaraman sheep, Kangal Akkaraman sheep, Intracranial volume, Stereology.

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Akkaraman sheep breed is intensively raised in Turkey, especially in Central Anatolia, and constitutes almost half of the current sheep population (Akçapınar 2000). Kangal Akkaraman sheep breed, which was previously considered a variety within the Akkaraman sheep breed, one of the indigenous breeds of Turkey, was registered as a separate breed with the published Communiqué (Oğrak et al 2014). Studies on the cranial cavity are crucial for understanding clinical and veterinary anatomy since the skull houses critical organs including the brain and portions of the digestive and respiratory systems (Miller et al 2013). Researchers that study sexual dimorphism and taxonomy categorization, for example, often use cranial measures (Adebisi 2009, Evin et al 2008). The intracranial volume value may be used to assess the change in volume after craniofacial procedures, to better understand the pathophysiology of intracranial pressure issues, and to help establish treatment plans for patients (Abbott et al 2000). Similar to plant seed, anthropometry, planimetry, stereology, and computerized tomography (CT) techniques, cerebral volume may also be determined using MRI and CT techniques (Acer et al 2007, Mendoza et al 2014, Choudhary et al 2015, Kamdar et al 2009, Ertekin et al 2016). Stereology is a method that allows the volume of complex three-dimensional objects to be estimated using two-dimensional sections (Mayhew and Gundersen 1996). Cavalieri's principle is a technique that uses equally random parallel sections to determine the overall volume of organs and structures with irregular shapes (Canan et al 2002). Imaging techniques like CT and MRI can also be utilized with this method (Odacı et al 2005).

Although there are studies in the literature on intracranial volume calculations for some animal species (Karimi et al 2011, Rodrigues et al 2017, Logan and Clutton-Brock 2013, Choudhary and Singh 2015); however, there is no study on Akkaraman and Kangal Akkaraman sheep. The objective of this study was to use 3D models created from stereological and CT sections to quantify the intracranial volume in Akkaraman and Kangal Akkaraman sheep. Based on the results, a statistical calculation was made to determine how different the breeds and procedures were. By contrasting the findings of this investigation with the intracranial volumes found in research done on other breeds, it also hoped to add to the scant literature in this area.

#### **Material and Methods**

In this study, 20 male 6-8 months old sheep (10 Akkaraman and 10 Kangal Akkaraman sheep) skulls were used. The study was approved by the Selçuk University Animal Experiments Local Ethics Committee (Approval no:2023/059). The heads of the sheep were scanned using 64-slice Multi-Detector Computed Tomography (MDCT) at 0.625 mm slice thickness.

Using pictures stored in the DICOM (Digital Imaging and Communications in Medicine) format, 3D Slicer software was used to reconstruct the skull. Using the CT section data in the 3D Slicer program, the intracranial cavity's limits were established. Sections of the designated region were used to construct 3D models. Figure 1 shows the volumetric values of the models that were built. The stereology method was used to identify the edges of the skull, and 12 slices from each sheep were taken from the CT sections of these areas using a systematic random sample technique. The acquired slice images were computed using the Cavalier principle and counted at 81 mm2 intervals using the "Grid" option in the "Image]" program. Three separate times, the identical process was carried out for the purpose of calculating (Figure 2). Intracranial volumes were estimated using the following equation:

 $Vtotal = \sum P \times [SU \times d/SL] 2 \times t$ 

 $\Sigma$ P: Total number of points on the surfaces of the slices

SU: the length represented by the scale showing the image magnification

SL: the length of the scale in the image measured with a ruler or caliper

t: Section thickness

d: the distance between two points on the dotted area measurement ruler.

The coefficient of error (CE) was estimated using the following equation:

$$CE(V) = \frac{1}{\sum p} \left( \frac{1}{12} (3a + c - 4b) \right)^{\frac{1}{2}} a = \sum_{i=1}^{m} P_i \cdot P_i \quad b = \sum_{i=1}^{m} P_i \cdot P_i + 1 \quad c = \sum_{i=1}^{m} P_i \cdot P_i + 2$$

#### Statistical Analysis

Data obtained from volume measurement were analyzed with the t-test in independent groups, and comparison between methods was analyzed with the Bland-Altman analysis in the SPSS 26.0 package program.

#### Results

In the study, when the intracranial volume was calculated using the stereological methodology, the intracranial volume of the Akkaraman sheep was found to be 116.48±9.52 cm<sup>3</sup>, and the intracranial volume of the Kangal Akkaraman sheep was found to be 132.06±8.08 cm<sup>3</sup>. When the same intracranial volume was measured using 3D modeling created from cross-sectional images obtained via CT, the intracranial volume of the Akkaraman sheep was found to be 118.43±9.67 cm<sup>3</sup>, and the intracranial volume of the

measurement is 0.027.						
Method	Breed	n	Mean	SD	P-value (Between the method)	P-value (Within the method)
Stereology	Akkaraman Sheep	10	116.4865	9.52609	P>0.05	P<0.01
	Kangal Akkaraman Sheep	10	132.0617	8.08767		
Computed Tomography	Akkaraman Sheep	10	118.4377	9.67988		
	Kangal Akkaraman Sheep	10	134.3685	7.24692		

Table 1. Estimation of intracranial volume (cm<sup>3</sup>) in Akkaraman and Kangal Akkaraman sheep using Stereological and Computed Tomography methods. The Coefficient Error (CE) value for stereological volume

Kangal Akkaraman sheep was found to be 134.36±7.24 cm<sup>3</sup>. Kangal Akkaraman sheep were found to have higher total intracranial volume than Akkaraman sheep (P<0.01). According to Bland Altman analysis, no statistical difference was observed between the methods. Despite the models produced by computed tomography had a higher total mean

intracranial volume, there was no statistically significant difference between the two techniques (P>0.05) (Figure 3). Table 1 shows the total intracranial volume data of Akkaraman and Kangal Akkaraman sheep by computed tomography and stereological methods.



Figure 1. Example of calculating the volume of a three-dimensional intracranial model created using the 3D Slicer software.

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Figure 2. Example of volume calculation using a point-counting grid with ImageJ software from Computed Tomography images.

#### Discussion

The intracranial cavity is an anatomical structure crucial for diagnosing significant diseases. One of the frequently preferred methods for examining the intracranial space is the use of CT models (Demircioğlu et al 2021b). In addition to calculating volume from CT or MRI sectional images, the Cavalieri principle can be used to determine the volume of substances with regular or irregular boundaries (Yılmaz and Tuğrul 2019). In our study, the volume of three-dimensional images created from sections obtained using the Cavalieri principle and CT imaging, both of which are preferred methods for calculating intracranial volume was calculated through specialized software. The results were compared with both inter-method and inter-group statistical methods. According to these results, the mean intracranial volume of Kangal Akkaraman sheep was found to be statistically significantly larger than that of Akkaraman sheep (P<0.01). A statistical comparison of intracranial volumes calculated using the stereological method and CT models revealed a high level of agreement between the two methods (P>0.05). In the literature review, a study conducted on 20 male and 20 female Kıvırcık sheep, the intracranial volume in male Kıvırcık sheep was found to be 136.10±17.281 cm3 and it was seen that no difference in cranial volume was found between the sexes (Ömer and Alpak 2012). Similarly, no significant gender difference observed in the research conducted on male and female Hamdani sheep (Koçyiğit et al 2024). In a study measuring intracranial volume in female

and male goats, no significant difference was found between the sexes (Rodrigues et al 2017). The cranial volume of Mehraban sheep selected without gender discrimination was calculated as 130.86±11.55 cm<sup>3</sup> using rice grains (Karimi et al 2011). Similarly, in a study conducted without gender differentiation, the cranium volume of Kagani goats was found to be 113±0.84 cm<sup>3</sup> (Sarma 2006). According to the studies on different species without gender discrimination, intracranial volumes were calculated as 0.172 L for Sitatunga, 0.314 L for Malayan tapir, 0.171 L for Chital gazelle, 0.162 L for Muntiacus muntjac, 0.197±0.013 L for Grant gazelle and 0.145±0.009 L for sheep, respectively (Chanpanitkitchote et al 2015). The cranial cavity of Saanen goats calculated using CT and stereological method was found to be 423±48.2 cm3 (Tohidifar et al 2020). In antelopes, the cranial cavity was found to be 106.33 cm<sup>3</sup> in males and 109.33 cm<sup>3</sup> in females, with no statistically significant difference between the sexes (Choudhary and Singh 2015). In red deer, endocranial volume was measured using both CT and bead methods, yielding results of  $370 \pm 39 \text{ cm}^3$  with the CT method and 362 ± 37 mL with the bead method, with a strong correlation observed between the two techniques (Logan and Clutton-Brock 2013). In gazelles, intracranial volumes were measured using Cavalieri's principle and 3D CT models, revealing a strong correlation between the two methods with no significant statistical difference between them (Demircioğlu et al 2021a).

Similar findings were achieved using both approaches in this investigation, and there was no statistically significant difference between computed tomography and stereology in the calculation of the cranial cavity. Therefore, future research in these areas can employ either approach. It is anticipated that this work will contribute to the body of knowledge on intracranial space and inform therapeutic strategies.

#### Conclusion



## Figure 3. Results graph based on Bland-Altman statistical analysis of data obtained using Stereological and Computed Tomography methods.



In conclusion, the intracranial volumes of Akkaraman and Kangal Akkaraman sheep were calculated using CT sections with the stereological method and with the help of a program from a 3D model. Compatibility between these two methods was investigated and the advantages/disadvantages of both methods were determined. Creating a 3D model from CT images is a long process. Therefore, measuring intracranial volume by creating a 3D model is time-consuming, but it is not necessary to deal with formulas after the model is created. Similar results can be obtained in less time with the stereological technique. On the other hand, the formulas required to apply the stereological technique can be confusing and difficult. We hope that both techniques will be useful for surgical procedures and disease diagnoses in veterinary medicine.

#### **Conflict of Interest**

Authors declares that there are no conflicts of interest related to the publication of this article.

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#### **Author Contributions**

Motivation/Concept: HBE, KB; Design: HBE; Control/ Supervision: KB; Data Collection and/or Processing: HBE; Analysis and/or Interpretation: HBE; Literature Review: HBE; Writing the Article: HBE; Critical Review: KB, HBE

#### **Ethical Approval**

The study was approved by the Selçuk University Animal Experiments Local Ethics Committee 25.05.2023, 2023/059 Number Ethics Committee Decision

106