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Anatomical and morphometric study of goat middle ear ossicles *(Capra aegagrus hircus)*

[*Estudo anatômico e morfométrico dos ossículos da orelha média de caprinos (*Capra aegagrus hircus*)*]

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ABSTRACT

The social and economic roles of goat farming in Northeastern Brazil, allied to the fact that the use of goat middle ear ossicles for research and human ear surgery training has not yet been proposed, justify the study of their applicability as an experimental model. The middle ears of 19 goats (*Capra aegagrus hircus*) from the bone collection of the Laboratory and Didactic Anatomy Museum of Domestic and Wild Animals of the Federal University of Vale do São Francisco (UNIVASF) were dissected. The malleus, incus, and stapes were evaluated regarding their macroscopic morphology and biometry (length, width, and height). Ossicle morphology was similar to sheep, human, and bovine morphology. The malleus was 1.3 times heavier and 2.2 times longer than the incus, and 9.0 times heavier and 3.7 times longer than the stapes. The size relationship was positive between the stapes and the malleus and negative between the stapes and the incus. It is concluded that the middle ear size and the anatomical similarities with human ossicles make goats a useful model for experimental scientific studies, reconstructive surgery practice of the ossicular chain, and human ear surgery training.

Keywords: malleus, incus, stapes, macroscopic anatomy, morphometry

RESUMO

Tanto o papel social quanto o econômico da caprinocultura na região Nordeste do Brasil, somados ao fato de que o uso de ossículos da orelha média de caprinos para estudos e treinamento cirúrgico otológico humano ainda não foi proposto, justificam o estudo de sua aplicabilidade como modelo experimental. Foram dissecadas as orelhas médias de 19 caprinos (Capra aegagrus hircus), *provenientes do ossuário do Laboratório e Museu Didático de Anatomia dos Animais Domésticos e Silvestres - Universidade Federal do Vale do São Francisco. Martelos, bigornas e estribos tiveram a morfologia macroscópica e a biometria (comprimento, largura e altura) avaliadas. A morfologia dos ossículos assemelhou-se a de ovinos, humanos e bovinos. O martelo foi 1,3 vez mais pesado e 2,2 vezes mais comprido que a bigorna e 9,0 vezes mais pesado e 3,7 vezes mais comprido que o estribo. A relação de tamanho entre o estribo e o martelo foi positiva, e entre o estribo e a bigorna negativa. Conclui-se que o tamanho da orelha média e as semelhanças anatômicas com os ossículos humanos tornam os caprinos um modelo útil para estudos científicos experimentais, prática cirúrgica reconstrutiva da cadeia ossicular e treinamento cirúrgico otológico humano.*

Palavras-chave: martelo, bigorna, estribo, anatomia macroscópica, morfometria

INTRODUCTION

The human ear is essential for communication as it allows capturing and interpreting information from the environment (Bertachini and Gonçalves, 2002), contributing to the language, cognitive and biopsychosocial development, and the intelligibility of the spoken message (Kesser *et al.*, 2013). Therefore, understanding the hearing mechanism and simulating pathologies to anticipate their auditory consequences and

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possible forms of treatment has been the subject of studies.

In the middle ear, the malleus, incus, and stapes ossicles form a mobile chain limited by the tympanic membrane and the oval window (Fragoso *et al.*, 2014), its main purpose being the transmission of sound vibrations to the liquid medium of the inner ear and entrance protection from excessive sound vibrations (Zemlin, 2005). Conductive hearing loss occurs due to outer and middle ear problems that prevent sounds from reaching the inner ear and may be caused by the presence of fluid in the middle ear (otitis media) or damaged (Thomeer *et al.,* 2012) or malformed ossicles (Bartel-Friedrich and Wulke, 2007; Esteves *et al.,* 2014).

The difficulty in obtaining human material, especially in studies aimed at the microscopic magnification of delicate structures such as the middle ear ossicular chain, has required the use of animal models in research and ear surgery training, chosen according to the purpose of the study, anatomical similarity, commercial availability, and captivity conditions, among others (Albuquerque *et al.,* 2009; Reis *et al.,* 2017). In general, these studies use guinea pigs, rats, mice, hamsters (Schanaider and Silva, 2004; Albuquerque *et al.,* 2009; Reis *et al.,* 2017), chinchillas, cats, dogs, and monkeys, among others (Reis *et al.*, 2017), due to their ease of handling and similarity with the human ear. Although all mammals have these three ossicles, differences in the middle ear shape and size can interfere with some results (Péus *et al.,* 2020), motivating the search for an experimental model that gathers all desirable features.

The significant expression of goat farming in Northeastern Brazil, which concentrates 90% of the national goat herd (Pesquisa…, 2017), in addition to the important social and economic role that it performs to the rural population and the regional economic structure (Nogueira Filho and Kasprzykowski, 2006; Costa *et al.*, 2008), and the fact that the use of goat ossicles for research and human surgery training has not yet been proposed justify the study of their applicability as an experimental model.

Thus, the present study aimed to describe the macroscopic anatomy and morphometry of goat *(Capra aegagrus hircus*) middle ear ossicles to complement pre-existing interspecific studies and assess their viability as an experimental model for scientific studies and reconstructive surgery practice of the human ossicular chain.

MATERIAL AND METHODS

Nineteen adult goat skulls of unknown breed and sex were used in the study. The material came from the bone collection of the Laboratory and Didactic Anatomy Museum of Domestic and Wild Animals of the Agricultural Sciences Campus (CCA) of the Federal University of Vale do São Francisco (UNIVASF), located on Highway BR-407, Km 12, Lote 543, Irrigation District Senator Nilo Coelho (C1 District).

The skulls were skinned, defleshed, and macerated using only water, resulting in the removal of soft structures and preservation of the bones only, which were cleaned and bleached. Subsequently, the temporal bone structures in both antimeres were fractured in the proximal portion of the external auditory meatus using hemostatic forceps and a Mayo-Hegar needle holder to allow access to the tympanic cavity, which occupies the petrous portion of the temporal bone. The hypotympanum was opened to allow visualization of the middle ear structures. The head of the malleus was thus highlighted and, after fracturing the tympanic bulla, also the stapes and the incus.

The malleus, incus, and stapes were weighed with an electronic analytical balance (Marte® AY220), and their length, width, and height were measured with a digital millimeter caliper (Pantec®). Total malleus length was defined as the distance from the tip of the head (uppermost part) to the tip of the manubrium (lowermost part); malleus width was defined as the distance from the joint surface to the transverse lamina; malleus height was defined as the distance from the tip of the head to the tip of the anterior process (Fig. 1- A). Total incus length was determined as the distance from the tip of the short process (uppermost part) to the tip of the long process (lowermost part); incus width was determined as the distance from the tip of the short process to the joint surface; incus height was determined as the distance from the center of mass to the joint surface (Fig. 1- B). Finally, total stapes length was defined as the distance from the tip of the head (uppermost part) to the tip of the footplate (lowermost part); stapes width was defined as the distance from one end of the footplate to the other; stapes height was defined as the distance from the lowermost part of the rostral crus to the caudal end of the footplate (Fig. 1- C).

Biometric data (mean±standard deviation) were tabulated in Microsoft Excel and analyzed by Student's t-test ($p<0.01$) and Pearson's correlation coefficient (Assistat 7.6 beta).

RESULTS

The malleus was mediolaterally positioned and consisted of a head, a neck, and a manubrium. The head was well-developed, and its joint surface and notch articulated with the surface of the body of the incus. The head showed a prominent notch and a diameter larger than the neck, which was convex. The lateral process extended from the distal part of the neck, followed by the manubrium, which occupied most of the malleus. The manubrium was the largest part of the malleus and showed a gradual reduction in diameter (Fig. 2- A).

The incus was located between the malleus and the stapes, and two processes emerged from its body: a short process and a long process. The long process was almost three times larger than the short process, and in its medial surface was the lenticular process, where the incus articulated with the head of the stapes. There was a concave depression on the dorsal surface of the body where the head of the malleus was articulated (Fig. 2- B).

The stapes consisted of a head, a neck, two crura, and a footplate. The head was small, flattened, and gradually increased in diameter toward the footplate. The neck was well-developed, and from it emerged two crura: a caudal and a rostral one, merging with the footplate. The rostral crus was slightly tilted and longer than the caudal crus. The footplate was flat and long, articulating with the oval window (Fig. 2- C).

When comparing biometric variables, the malleus was the ossicle with the largest mass and length, the incus was the tallest ossicle, and the stapes showed the smallest mass, length, height, and width. The malleus was 1.3 times heavier and 2.2 times longer than the incus (Table 1).

Table 2 shows Pearson's correlation coefficient (r) between the biometric data of the malleus, incus, and stapes of goats. The correlation analysis of the malleus showed a strong positive correlation ($P<0.01$) between malleus length and height and a moderate positive correlation ($P<0.05$) of malleus mass with malleus length and height. There was a strong positive correlation ($P<0.01$) between incus length and height and a strong positive correlation ($P<0.01$) between stapes length and height, in addition to a moderate negative correlation ($P<0.05$) of stapes mass with stapes length and height. In the three ossicles, a strong positive correlation was observed between length and height.

When analyzing the malleus and the incus, a moderate negative correlation was observed ($P<0.05$) between malleus mass and incus mass, and a moderate positive correlation ($P<0.05$) between malleus mass and incus width. In addition, there was a strong positive correlation ($P<0.01$) between stapes width and malleus length and height, and a moderate positive correlation ($P<0.05$) between malleus mass and stapes width and between malleus width and stapes length, width, and height. There was a strong negative correlation ($P<0.01$) between the length and height of both incus and stapes, between incus length and stapes height, and between incus height and stapes length. Finally, there was a moderate negative correlation ($P<0.05$) between stapes mass and malleus and incus width

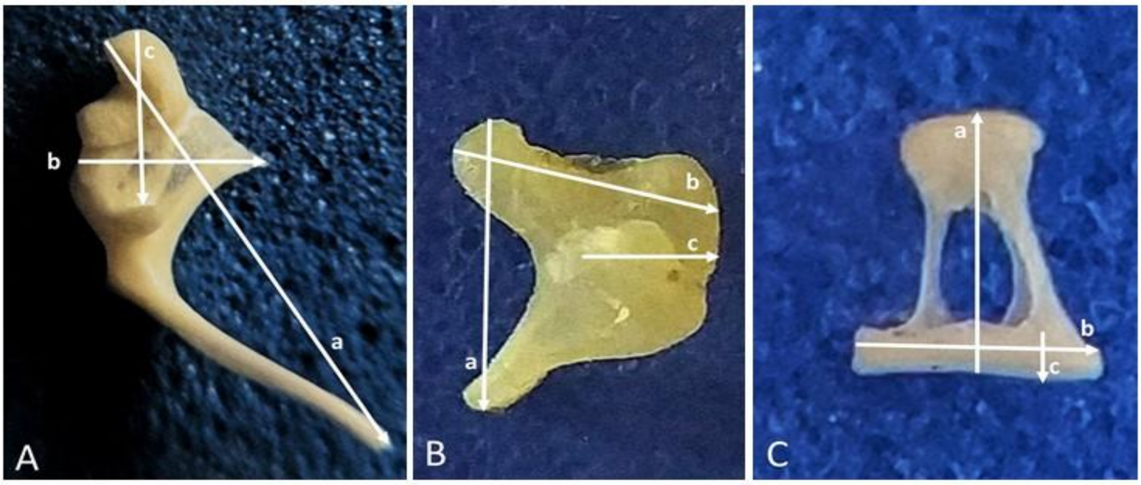

Figure 1. Biometric analysis of the malleus (A), incus (B), and stapes (C) from sheep of unknown breed measured as (a) length, (b) width, and (c) height.

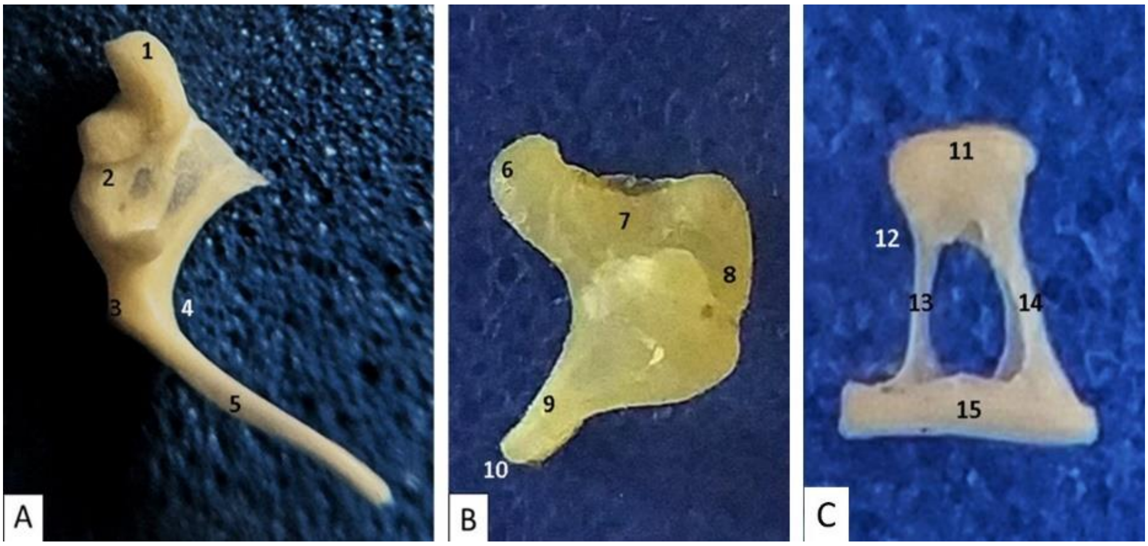

Figure 2. Morphology of the malleus (A), incus (B), and stapes (C) of goats of unknown breed. Petrolina-PE, 2020. Note in A: head (1); joint surface (2); lateral process (3); neck (4); and manubrium (5) of the malleus; in B: short process (6); body (7); joint surface (8); long process (9); and lenticular process (10) of the incus; and in C: head (11); neck (12); caudal crus (13); rostral crus (14); and footplate (15) of the stapes.

Table 1. Biometric data (mean±standard deviation) of the malleus, incus, and stapes of crossbred goats. Petrolina (PE), 2020

Ossicle / Aspects	Malleus	Incus	Stapes
Mass (mg)	13.28±5.62[a]	10.65±1.53[a]	1.47±0.28[b]
Height (mm)	1.67±0.40[b]	1.99±0.24[a]	1.03±0.12[c]
Width (mm)	3.07±0.45[a]	3.37±0 52[a]	2.07±0.23[b]
Length (mm)	8.23±1.04[a]	3.77±0.26[b]	2.24±0.19[c]

[a,b,c]Values in the same row differ by Student's t-test ($p<0.01$).

Table 2. Pearson's correlation coefficient(r) between the biometric data of the malleus, incus, and stapes of crossbred goats. Petrolina (PE), 2020

	ML	MW	MH	IM	IL	IW	IH	SM	SL	SW	SH
MM	0,60*	-0,49^{ns}	0,60*	-0,77*	0,12^{ns}	-0,51*	0,12^{ns}	-0,31^{ns}	-0,13^{ns}	0,60*	-0,13^{ns}
ML	----	0,48^{ns}	1,00**	-0,43^{ns}	0,01^{ns}	-0,39^{ns}	0,01^{ns}	0,22^{ns}	0,08^{ns}	0,89**	0,08^{ns}
MW	----	----	0,48^{ns}	-0,49^{ns}	-0,35^{ns}	-0,26^{ns}	-0,35^{ns}	-0,71*	0,58*	0,67*	0,58*
MH	----	----	----	-0,43^{ns}	0,01^{ns}	-0,39^{ns}	0,01^{ns}	0,22^{ns}	0,08^{ns}	0,89**	0,08^{ns}
IM	----	----	----	----	0,39^{ns}	0,13^{ns}	0,33^{ns}	0,44^{ns}	-0,11^{ns}	-0,66*	-0,11^{ns}
IL	----	----	----	----	----	-0,45^{ns}	0,89**	0,49^{ns}	-0,86**	-0,41^{ns}	-0,86**
IW	----	----	----	----	----	----	-0,29^{ns}	-0,17^{ns}	0,01^{ns}	-0,26^{ns}	0,01^{ns}
IH	----	----	----	----	----	----	----	0,49^{ns}	-0,86**	-0,41^{ns}	-0,86**
SM	----	----	----	----	----	----	----	----	-0,52*	-0,13^{ns}	-0,53*
SL	----	----	----	----	----	----	----	----	----	0,43^{ns}	1,00**
SW	----	----	----	----	----	----	----	----	----	----	0,43^{ns}
SH	----	----	----	----	----	----	----	----	----	----	----

**Strong correlation ($P<0.01$); *Moderate correlation ($P<0.05$); ^{ns}Non-significant correlation.

DISCUSSION

The lack of scientific studies describing the macroscopic anatomy and morphometry of goat middle ear ossicles restricted this subject to comparisons with other domestic and wild species and with humans. There were morphological similarities with sheep and bovines (Getty, 1986; Péus *et al.,* 2020**)**, guinea pigs and adult rats (Albuquerque *et al.*, 2009), paca (Martins *et al.*, 2015), and humans (Costa, 2008; Sobotta, 2018).

The differences observed in the malleus comprised a larger prominence of the head notch in goats and sheep as compared to bovines, the neck as the thickest part, and the junction with the head with a smaller size in sheep (Getty, 1986). The goat incus was similar to the sheep incus with regard to process length as the long process was almost three times larger than the short process and due to the presence of the lenticular process (Péus *et al.,* 2020**)**. The manubrium was thinner and longer in goats than in adult rats; the lenticular process of the incus was not bent toward the short process; the stapes had a triangular shape, and their rostral and caudal crura were longer. In turn, guinea pigs had a smaller and thicker manubrium; the incus was fused to the malleus, and the stapes had a triangular shape similar to goats (Albuquerque *et al.,* 2009). Pacas showed similarity regarding the triangular shape of the stapes (Martins *et al.,* 2015).

The malleus head in humans showed a round shape, a prominent lateral process, and a smaller and less narrow manubrium (Sobotta, 2018). The short process of the incus had a triangular shape (Costa, 2008; Sobotta, 2018), and the stapes was very similar to that of goats, except for the cylindrical-discoid and flattened shape from the uppermost to the lowermost part of the head, the greater range of the semicircle formed by the crura, and the irregular and oval shape of the footplate (Costa, 2008; Sobotta, 2018). The similarity of the macroscopic anatomy of human and goat middle ears, allied to the advantageous preservation of access ways after the procedure, demonstrated the possibility of using goats as experimental models for human ear studies.

The goat malleus was lighter than the human malleus (22.40 to 30.42mg; Sim *et al.,* 2007; Costa, 2008; Gan *et al.,* 2010; Fragoso *et al.,* 2014) and similar or heavier than the sheep malleus (13.4mg; Péus *et al.,* 2020), whereas its length was similar to the human ossicle (8.11mm; Gan *et al.,* 2007; Dai *et al.,* 2007; Gan *et al.,* 2010) and longer than in sheep (7.88±0.51; Péus *et al.,* 2020). The goat incus was lighter than the human incus (24.2 to 32.0±5.9mg; Gan *et al*., 2007; Sim *et al.,* 2007; Costa, 2008; Dai *et al.,* 2007; Gan *et al.,* 2010) and heavier than the sheep incus (7.73 mg; Péus *et al.,* 2020), whereas its length was smaller than in humans (6.3 to 6.64 mm; Gan *et al.,* 2007; Sim *et al.,* 2007; Costa, 2008; Dai *et al.,* 2007; Gan *et al.,* 2010). The goat stapes was lighter than the human stapes (1.93 mg to 3.2mg; Gan *et al.,* 2007; Sim *et al.,* 2007; Dai *et al.,* 2007; Gan *et al.,* 2010) and sheep stapes (1.75mg; Péus *et al.,* 2020), whereas the length was similar to the human ossicle (2.4 to 3.4mm; Gan *et al.,* 2007; Dai *et al.,* 2007; Gan *et al.,* 2010) and longer than in sheep (2.03±0.12mm; Péus *et al.,* 2020). These findings suggest important size similarities between the goat and human ossicular chain structures, which, allied to the ease of obtaining goats, represents an advantage for their use as an experimental model in research and human ear surgery practice.

The negative correlation observed between malleus mass and incus mass in goats differed from the positive correlation described in humans (Todd and Creighton, 2013; Sodhi *et al.,* 2017) but agreed with the observation that the malleus was 1.3 times heavier than the incus. This is a novel finding and agrees with the results observed in sheep, whose malleus is almost twice as heavy as the incus (Péus *et al*. 2020) but differed from two other species of the order Artiodactyla (*Camelus bactrianus* and *Bos taurus*), from Pinnipedia (Nummela, 1995), and from humans, whose incus was heavier than the malleus (Péus *et al.,* 2020). On the other hand, the negative correlation between stapes and incus size differed from humans, who showed a positive correlation between total incus length and total stapes height (Sodhi *et al.,* 2017).

When analyzing the mass of the malleus-incus complex, this structure represented from 38 to 51% in goats and from 33 to 45% in sheep of the mass of this complex in humans. Thus, except for the smaller stapes mass, the size of the goat ossicular chain was larger than that of sheep. Considering that the sheep middle ear can be used for human surgery training (Seibel *et al.,* 2006; Miller *et al.,* 2014; Larsson *et al.,* 2015; Pffner *et al.,* 2018), these results suggest that goats could be an experimental model as good as or better than sheep for middle ear manipulation during microdissection, with the additional advantage of having similar malleus and stapes lengths to those of humans. However, there are anatomical differences between the middle ears of humans and goats that may require further studies regarding their interference with surgical results.

In humans, the fact that malleus length was 1.3 times longer than stapes length implies that the action of a small driving force in the tympanic membrane results in a force 1.3 times greater on the oval window and a sound intensity of approximately 2 decibels (dB) (Wada, 2007). When establishing the same relationship in goats, a force 3.7 times greater is obtained, in addition to 4 dB of sound intensity, which seems to give an additional mechanical advantage to the goat lever system as this would have twice the capability of the human mechanism for sound amplification.

CONCLUSION

This study provides anatomical and quantitative data on the middle ear ossicles of goats, which are useful to create a middle ear model of this species. The size and anatomical similarities with the human middle ear, the commercial availability, economic viability, and the compliant behavior in captivity make goats a useful experimental model for scientific studies, reconstructive surgery practice of the ossicular chain, and human ear surgery training. Furthermore, malleus size relative to incus size gives the lever system an additional mechanical advantage and greater capability for sound amplification.

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