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Evaluation of the middle ear in water buffaloes (*Bubalus bubalis*) by gross anatomy and cone-beam computed tomography

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Background: *The purpose of this study was to provide a description of gross middle ear morphology in water buffaloes, augmented with additional data on the osseous structures of middle ear derived from cone-beam computed tomography (CBCT).*
Materials and methods: *Skulls of 10 young adult male water buffaloes were used to examine their middle ears.*
Results: *Anatomical features noted included the presence of tympanic cells in the tympanic bulla, the location of malleus head and neck, and all of incus in the dorsal epitympanic recess, the oval tympanic membrane, absence of a prominent notch on the articular surface of malleus, positional variations of the lateral process of malleus relative to the muscular process and muscular process relative to the rostral process of malleus, absence of complete coverage of the articular facet of malleus head by incus body, and presence of the lenticular process of incus. In CBCT images, the osseous part of external acoustic meatus, the petrous part of temporal bone and the details of the ossicles were seen, except for stapes.*
Conclusions: *Although tympanic membrane, malleus and stapes of water buffaloes are similar to those of ox, the incus of water buffaloes is more similar to that of goats. The heaviest ossicles among the ruminants studied belonged to water buffaloes; the mean length of malleus head and neck, total length and width of incus body as well as length of stapes head were greatest in water buffaloes too. The auditory ossicles of water buffaloes show 'transitional type' morphological characteristics. These features suggest a relatively wide frequency range of hearing, but not one biased towards especially low or especially high frequencies.* (Folia Morphol 2021; 80, 1: 177–185)

Key words: anatomy, auditory ossicles, cone-beam computed tomography, middle ear, morphometry, water buffalo

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INTRODUCTION

The middle ear cavity is an irregular space within the temporal bone that is filled with air. It contains the malleus, incus, and stapes, which form a chain and serve to convey vibrations from the tympanic membrane (TM) across the cavity to the internal ear [11]. Many studies on the middle ear have been conducted from morphological, functional, and surgical viewpoints, with physiological experiments typically having been performed on relatively small laboratory animals such as gerbils, guinea pigs and cats. There is considerable variation in middle ear architecture among the many species studied [7, 15, 20, 21]. Body size, phylogeny, style of life, ecology, and acoustic environment can all be reflected in aspects of ear morphology [29].

Recently, there has been more interest in the middle ear of larger animals such as sheep and pig, which potentially represent better models of the human middle ear [18, 28]. However, the gross morphology of middle ear of ox, sheep, and goat is not described or illustrated in any detail in most veterinary anatomical textbooks, atlases and dissection guides [5, 8, 14, 24, 25, 32]. Only Getty [11], in his veterinary anatomical textbook, included detailed comparative descriptions of this area, but did not present any schematic pictures and photographs. There are a few descriptive anatomical accounts of sheep and ox ears [4, 34, 35], but there remains much to be clarified regarding the comparative anatomy of ruminant auditory systems.

Cone-beam computed tomography (CBCT) acquires data volumetrically, providing accurate, three-dimensional (3D) radiographic imaging which is ideal for the assessment of osseous structures of the maxillofacial region at sub-millimeter resolution [17]. This has proved to be an excellent technology for the identification and description of the normal anatomy of middle ear structures in veterinary research [31]. CT studies on the normal anatomy of middle ear structures have been conducted in sheep [33], and horses [3].

Water buffaloes (*Bubalus bubalis*) are placed in the family Bovidae, within the order Artiodactyla. Some archeological evidence suggests that water buffaloes were first domesticated in Iran and migrated to southern Europe from this region [23]. The Khuzestan ecotype of Iranian water buffaloes are likely to be the biggest buffalo breed in the world [22], the morphological appearance of which differs from that of Mediterranean water buffaloes [23]. No description of the middle ear of water buffaloes seems to have been published. The goals of this study were, therefore, (1) to provide a detailed description of the middle ear of water buffaloes, (2) to determine the similarities and differences between water buffaloes ears and those of other ruminants, and (3) to describe the osseous structures of the middle ear of water buffaloes using CBCT.

MATERIALS AND METHODS

Skulls of 10 young adult male water buffaloes, without any external abnormality or pathology, were collected from a local slaughterhouse in Ahvaz, southwest Iran. The age of animals, as estimated from the eruption of teeth [9], ranged between 2 to 3 years. The animals' live weight (316–383 kg) was estimated based on carcass weight (190–230 kg). There was no need of approval from the Local Ethical Committee as the animal skulls were collected from the slaughterhouse. The maceration of skull and the dissection of tympanic cavity were performed according to the procedures of Nummela [27], and Martonos et al. [19], respectively. Afterwards, the following measurements for separated auditory ossicles were taken according to Martonos et al. [19]. Malleus: total length (TL), length of the manubrium (LM), head diameter (HD), and head and neck length (HNL). Incus: total length (TL), body width (BW), length of short crus (LSC), and length of long crus (LLC). Stapes: total length (TL), head length (HL), and thickness of crura at the middle (TCM). All variables were measured three times by a single researcher using a digital calliper (150 mm, Mitutoyo, Japan) and presented as means and standard deviations. The masses of ossicles were measured to an accuracy of 0.001 g on laboratory scales (A&D Company, MA 3000, Japan). Photographs of the ossicles were taken under a stereomicroscope (Nikon, SMZ800, Japan) using a Canon digital camera (G9, Tokyo, Japan).

Cone-beam CT images were acquired using New Tom VGi scanner (New Tom GRsrl; Verona, Italy) with a field of view 12 cm $\times$ 8 cm at a 0.15 mm voxel resolution with the scanning parameter of 110 kVp, 9.18 mA (for left side) and 7.33 mA (for right side), and 5.4 seconds exposure time. CBCT data were evaluated in the dorsal and sagittal planes by an expert maxillofacial radiologist.

RESULTS

Tympanic membrane

The mean and standard deviation of rostrocaudal and dorsoventral dimensions of TM were 11.66 $\pm$ $\pm$ 0.63 mm and 9.12 $\pm$ 0.12 mm, respectively. The TM

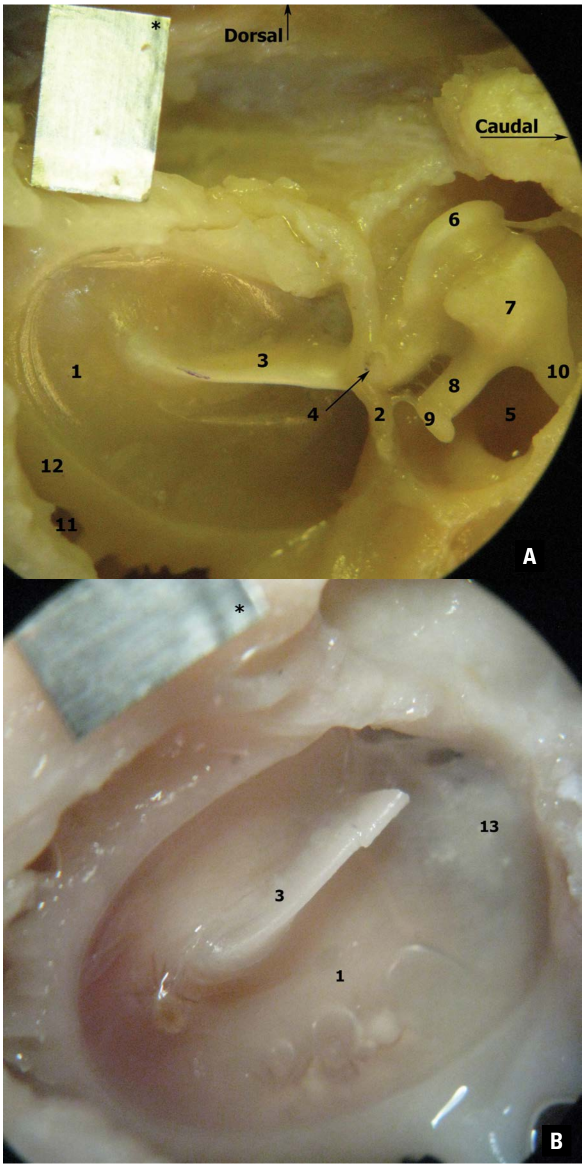

Figure 1. Medial aspect of right ossicles and tympanic membrane in water buffaloes (in situ); **A. B.** 1 — pars tensa; 2 — chorda tympani nerve; 3 — manubrium; 4 — muscular process; 5 — epitympanic recess; 6 — malleus head; 7 — incus body; 8 — long process; 9 — lenticular process; 10 — short process; 11 — tympanic cell; 12 — tympanic ring; 13 — pars flaccida; *Scale = 5 mm.

was made up of two major parts, the pars flaccida (PF) and pars tensa (PT). The PF, an irregular triangle, was small and extended from the surrounding parts of the tympanic incisures of squamous temporal bone to the lateral process of malleus (Fig. 1B). The shape of PT was a regular oval. The medial surface of TM appeared to be irregularly concavo-convex. The entire length of manubrium was embedded in PT. The manubrium almost in its middle was very strongly attached to the medial surface of PT (Fig. 1A, B).

Table 1. Means (mm) ± standard deviations of the ossicles (water buffaloes)

Malleus	**TL**	**ML**	**HNL**	**HD**
	11.07 ± 0.46	6.54 ± 0.6	5.73 ± 0.29	3.17 ± 0.4
Stapes	**TL**	**BL**	**HL**	**TMB**
	3.6 ± 0.57	2.7 ± 1.27	1.44 ± 0.33	0.7 ± 0.16
Incus	**TL**	**LCL**	**SCL**	**BW**
	5.74 ± 0.88	2.78 ± 0.16	2.74 ± 0.18	3.3 ± 0.22

TL — total length; ML — malleus length; HNL — head and neck length; HD — head diameter; BW — body width; HL — head length; BL — body length; TMB — thickness in the middle of bone; LCL — long crus length; SCL — short crus length

The epitympanic recess housed the head and neck of malleus, and all of the incus (Fig. 1A). The ventral part of middle ear cavity was divided into tympanic cells (see Fig. 5A).

Auditory ossicles

The morphometric data obtained from the ossicles are presented in Tables 1 and 2.

Malleus head. Caudally, the head was slightly convex and its edges rounded and somewhat pressed (Fig. 2A, C) and there was a small, shallow fossa just rostral to the root of the lamina on the lateral surface of head (Fig. 2A, C). The head had a concave facet on its caudomedial aspect for articulation with incus. The facet was divided by a shallow notch into dorsal and ventral parts (Fig. 2B).

Malleus neck. The curved neck was not very distinct below the head (Fig. 2A, B). The well-developed lamina was thin and irregularly triangular in shape: it originated from the dorsolateral surface of neck and extended rostrally, dorsally and laterally. The rostral process was a delicate projection in continuity with lamina, and was lateral to the large muscular process (Fig. 2A). The rostral process was attached by a ligament to the wall of tympanic cavity (not shown). There was a very distinct shallow fossa caudal to the muscular process on the mediodorsal side (Fig. 2B). The medial surface of neck, just rostral to the muscular process, was crossed by chorda tympani branch of facial nerve (Fig. 1A). The muscular process was a very well-developed and prominent spur which arose medially from the distal part of the neck (Fig. 1A, 2B).

Malleus manubrium. The manubrium had four sides. The lateral edge of manubrium was convex. It was attached to TM, whereas the medial edge of the manubrium was free. The rostrodorsal surface of the manubrium was slightly concave longitudinally

Table 2. Comparison of mean masses (mg) ± standard deviations of the ossicles (water buffaloes) with those obtained in various ruminates

	Malleus	Incus	Stapes
Present study	0.050 ± 0.030	0.048 ± 0.008	0.006 ± 0.001
Ox [11] [27]	0.032 ± 0.006 0.022	0.030 ± 0.005 0.026	0.005 ± 0.0005 0.002
Sheep [11] [27]	0.008 ± 0.001 0.007	0.008 ± 0.001 0.055	0.001 ± 0.0003
Goat [11]	0.010 ± 0.001	0.008 ± 0.001	0.001 ± 0.0004
Dromedary camel [21]	0.039 ± 0.080	0.030 ± 0.003	0.004 ± 0.0004
Bactrian camel [2] [27]	0.037 ± 0.02 0.037	0.032 ± 0.008 0.038	0.005 ± 0.0005 0.004
Human [12] [27]	0.023 ± 0.002 0.028	0.025 ± 0.002 0.033	0.003 ± 0.0006 0.002

Figure 2. The left malleus; **A.** Lateral view (*Scale = 5 mm); **B, C.** Medial view (*Scale = 1 mm) (water buffaloes); 1 — head; 2 — neck; 3 — lamina; 4 — rostral process; 5 — manubrium; 6 — lateral process; 7, 7′ — dorsal and ventral parts of articular facet; 8 — extremity; 9 — fossa; 10 — muscular process.

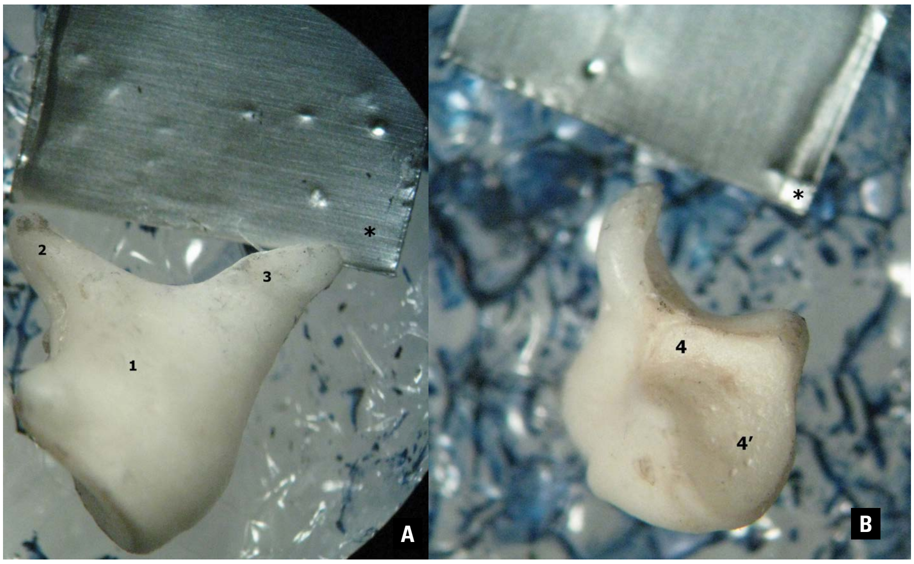

Figure 3. The left incus (water buffaloes); **A.** Lateral view; **B.** Articular surface; 1 — body; 2 — long process; 3 — short process; 4, 4′ — dorsal and ventral parts of articular facet; *Scale = 5 mm.

from body to tip, whereas the caudoventral surface was convex. The lateral process was a right-angled triangular protuberance which arose from the base of the manubrium at the same level as the muscular process on the opposite side (Fig. 2A, B).

The imaginary line extending between the anterior process of malleus and the short process of the incus, two points where the ossicles are tethered to skull, has been called the 'anatomical axis' [16]. The manubrium took an intermediate orientation relative to the anatomical axis, i.e. between perpendicular and parallel (Fig. 1A).

Incus. The medial surface of body was convex with a distinct oval facet (Fig. 3A), while the lateral surface was almost smooth (not shown). The shape of the articular facet was slightly saddle-shaped and occupied virtually the whole width of incus, and was divided by a ridge into two facets (Fig. 3B). The long process extended in a rostroventral direction. The lenticular process seemed to ossify with the extremity of long process. It was directed mainly caudally and formed a small, nodular projection. The pyramidal short process was oriented in a caudoventral direction. The incudo-mallear joint was easily separable (Fig. 1A).

Stapes. The head of stapes was convex. The stapes was quadrilateral in shape. The rostral crus of spates was slightly inclined rostrally, while the caudal crus was almost straight below the level of junction of caudal crus and neck at its caudomedial aspect, there was a small but prominent muscular process. The intercrural foramen was a regular oval in shape (Fig. 4A). Cross-section of the rostral crus was as a narrow semicircle near footplate, but caudal crus was somewhat appeared as an irregular c-shaped (Fig. 4C). Footplate was an irregular oval in shape (Fig. 4B), its extremities extending considerably beyond the limits of insertion of crura. However, it extended farther beyond the insertion of caudal crus than beyond the rostral crus. The well-developed labrum was thick at the extremities, especially at the caudal extremity. The caudal extremity was relatively pointed, whereas the rostral one was rounded when viewed from its vestibular aspect. The center of footplate was relatively thin and was slightly concave towards the vestibule (Fig. 4A). The footplate was placed in fossa ovalis leading to the oval window at its bottom (Fig. 4C).

CBCT anatomy. Osseous structures of water buffaloes middle ear were very well visualized with CBCT. The sagittal plane images provided excellent depictions of anatomical structures. No significant anatomic variations were noted between right and left sides of temporal bone. In the sagittal plane tomogram shown in Figure 5A, the body, short and long processes of incus, the head, neck, rostral process, and manubrium of malleus, incudo-mallear joint, tympanic bulla, tympanic cells and epitympanic recess were readily identified. In the dorsal plane tomogram shown in Figure 5B, the osseous part of external acoustic meatus, the bony rim supporting the tympanic membrane, part of the malleus, and the petrous part of temporal bone were seen clearly.

DISCUSSION

Tympanic membrane. Although the typical mammalian TM has a PT and smaller PF, the outline of PT differs among the species, from nearly circular to an

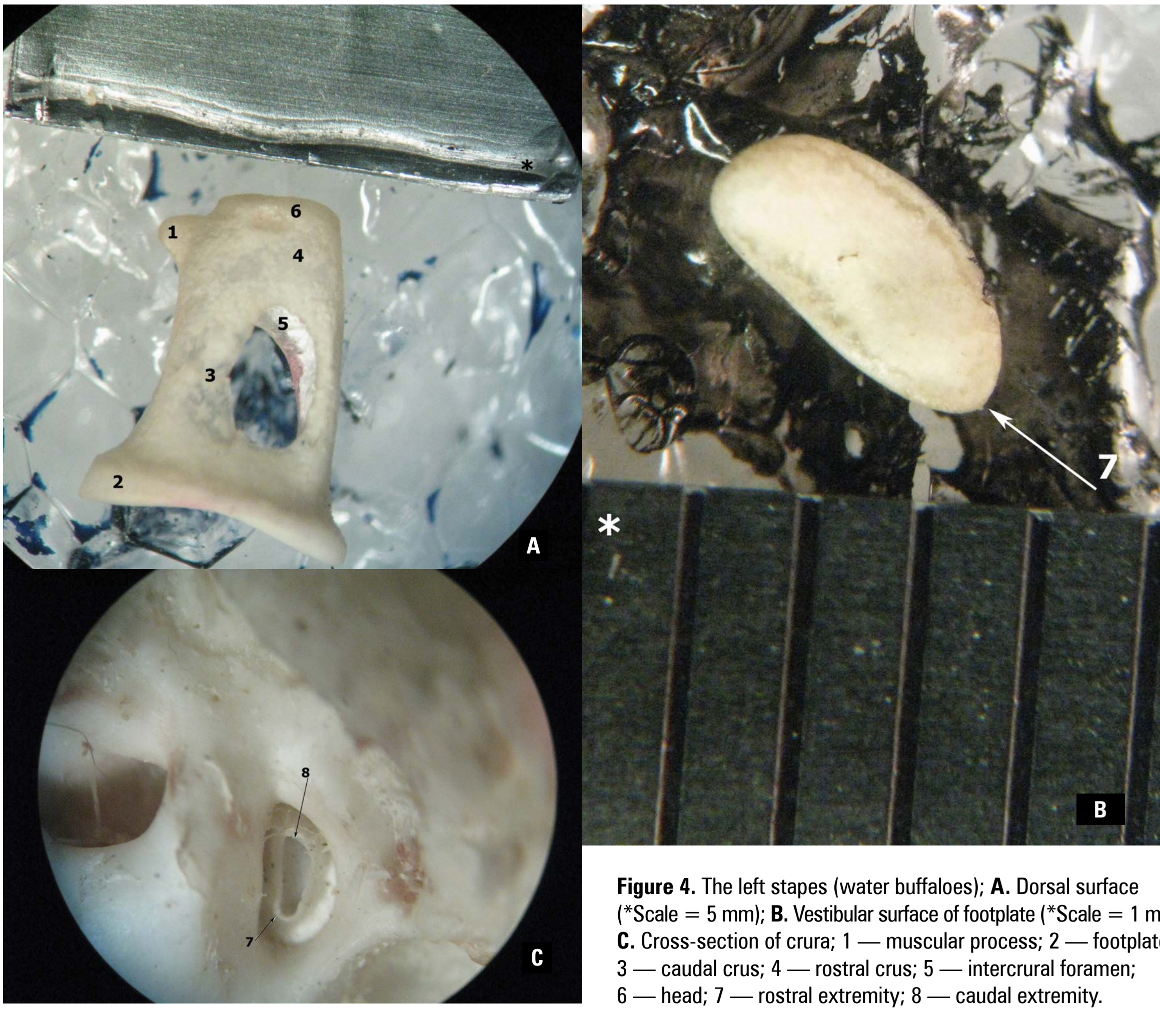

Figure 4. The left stapes (water buffaloes); **A.** Dorsal surface (*Scale = 5 mm); **B.** Vestibular surface of footplate (*Scale = 1 mm), **C.** Cross-section of crura; 1 — muscular process; 2 — footplate; 3 — caudal crus; 4 — rostral crus; 5 — intercrural foramen; 6 — head; 7 — rostral extremity; 8 — caudal extremity.

approximately elongated oval [6]. Like that of the ox, the PT of TM in water buffaloes was a regular oval, whereas the shape of PT in sheep and goats takes the form of a regular circle [11]. The shape of PF is more or less rectangular [11] or roughly triangular [35] in the ox and irregularly triangular in sheep and goats [11], as it was in water buffaloes. The TM is circular in one-humped camels [21].

Like the ox, sheep, dog, cat, and pig [6] the manubrium divides PT into asymmetrical dorsal and ventral sections in water buffaloes. Puria and Steele [30] hypothesize that a similar asymmetry in cats and humans ears could lead to a force differential on the manubrium, resulting in a rotation of malleus about its long axis. Because such a change in rotatory axis would reduce the moment of inertia, they suggest that this change in ossicular vibratory mode could improve the efficiency of sound transmission at higher frequencies.

As in the goat and ox [11], the entire length of manubrium in water buffaloes was embedded in PT, whereas in the sheep part of manubrium was superficially placed over PT [11]. The medial surface of PT on its central part was strongly convex in the ox or regularly convex in goats and sheep [11]. In water buffaloes, it was not convex; it appears to be irregularly concavo-convex.

The size of PF is very variable among mammals; out of humans, cats, dogs, the ox, sheep and rodents, the biggest PF belongs to the sheep [6]. Although we did not measure PF separately in water buffaloes, it is likely very similar in size to that of the ox as illustrated by Decraemer and Funnell [6]. In short, the TM of water buffaloes resembled that of the ox rather than those of goats and sheep.

Tympanic cavity. The epitympanic recess in water buffaloes was occupied by head and neck of the malleus, as well as all of the incus, whereas in small ruminants the head, neck and rostral process of malleus, along with its articulation with incus, are all situated in the recess [11]. In the ox only the head

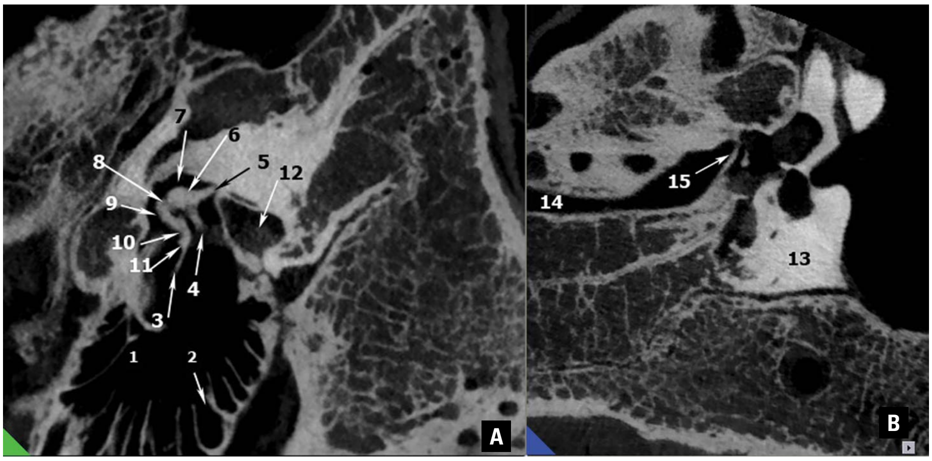

Figure 5. Sagittal (**A**) and dorsal (**B**) planes tomogram created by cone-beam computed tomography of right temporal bone (water buffaloes); 1 — tympanic cavity; 2 — tympanic cell; 3 — manubrium; 4 — long process of incus; 5 — short process of incus; 6 — incus body; 7 — epitympanic recess; 8 — incus-malleus joint; 9 — malleus head; 10 — malleus neck; 11 — rostral process of malleus; 12 — facial and vestibulocochlear nerves; 13 — petrosal part of temporal bone; 14 — external acoustic meatus; 15 — tympanic rim.

Table 3. Comparison of means (mm) ± standard deviations of the ossicles (water buffaloes) with those obtained in various ruminates

		Present study	Ox	Sheep	Goat	Dromedary camel	Bactrian camel [2]	Human [26]
Malleus	TL	11.07 ± 0.46	11.26 ± 0.61	8.28 ± 0.39	8.40 ± 0.41	10.25 ± 0.54	–	7.15 ± 0.31
	ML	6.54 ± 0.60	7.32 ± 0.13	5.56 ± 0.31	5.10 ± 0.55	7.60 ± 0.46	–	4.22 ± 0.35
	HNL	5.73 ± 0.20	3.17 ± 0.40	2.60 ± 0.21	2.60 ± 0.21	2.72 ± 0.17	–	4.85 ± 0.29
	HD	3.17 ± 0.40	1.76 ± 0.08	1.26 ± 0.16	2.18 ± 0.32	3.25 ± 0.17	1.60 ± 0.20	2.36 ± 0.21
Incus	TL	5.74 ± 0.88	6.20 ± 0.38	3.22 ± 0.13	3.10 ± 0.10	4.84 ± 0.20	–	3.14 ± 0.19
	BW	3.30 ± 0.22	2.92 ± 0.28	2.08 ± 0.08	2.14 ± 0.15	3.04 ± 0.15	–	–
Stapes	TL	3.60 ± 0.57	3.60 ± 0.57	2.72 ± 0.20	2.27 ± 0.20	4.12 ± 0.35	4.30 ± 0.60	3.12 ± 0.21
	HL	1.44 ± 0.33	1.42 ± 0.05	1.26 ± 0.11	1.07 ± 0.09	1.35 ± 0.17	–	–
	BL	2.70 ± 1.27	2.57 ± 0.09	2.08 ± 0.08	2.02 ± 0.05	3.25 ± 0.37	–	2.68 ± 0.27

Note: The author did not record the related parameters which are empty in Bactrian camel and human. TL — total length; ML — malleus length; HNL — head and neck length; HD — head diameter; BW — body width; HL — head length; BL — body length. The parameters are based on millimeters (mm).

of malleus and part of the incus [11] or the head of malleus and incus [4] are housed in the recess. In Bactrian camels, the malleus head and main part of incus are located in the recess [2].

The cellular divisions of the bullar cavity in water buffaloes resemble what has been described in the ox [1, 35]. This is not the case in other domestic ruminant species in veterinary anatomical textbooks [11, 25] nor in Bactrian camels [2]. According to Fleischer [10] and Plestilova et al. [29], similar bony septa which divide the middle ear cavities of several types of mammals are expected to change the resonance properties of such cavities, but experimental data are lacking.

Morphometry of ossicles. Differences in ossicle size in animals correlate with variations in their auditory range [13, 27]. Nummela [27] found that although there is a correlation between skull mass and ossicular mass among mammals in general; the ossicular mass cannot be reliably predicted from skull mass in mammals with large skulls. There are very few morphometric studies documented for ossicles of ruminants. Mohammadpour [21] reported masses and other measurements of the different parts and process of the ossicles in the ox, sheep, goat, and dromedary camel. Bai et al. [2] weighed all ossicles and measured malleus head and length of stapes in Bactrian camels. Nummela [27] reported average masses of ossicles in various mammals including Bactrian camels, cattle and sheep.

According to Table 2, the heaviest ossicles belonged to water buffaloes although it is not the largest animal among the reported animals. The mean values for HNL of malleus, TL and BW of incus, as well as HL stapes, were all greatest in water buffaloes among the other ruminants. However, the mean values of BL and TL of stapes were greatest in dromedary and Bactrian camels, respectively (Table 3). In the ox, the long crus was almost twice the length of short crus, whereas in the sheep the long crus was nearly three times longer than the short crus. The two crura of goats were almost equal in length [11], like water buffaloes, while high frequency hearing is limited by middle ear size and in particular ossicular mass [13].

Malleus head. Unlike small ruminants [11], caudally the malleus head is smoothly convex in the ox [11], as it was in water buffaloes. Wilkie [35] also reported that malleus head has very pronounced features in the flatness of head caudally.

Unlike the ox and water buffalo, the notch on the articular surface of head is very prominent in goat and sheep [11], which is the most striking difference among the mallei of these animals.

The articular surface of malleus head was covered by the incus body completely in the ox [11], while it was covered partly in goat and sheep [11], as it was in water buffaloes.

Malleus neck. As in the ox [11, 35], the curved neck was not very distinct in water buffaloes, whereas in the sheep the neck was the thickest part [11].

Malleus processes. The best-developed muscular process belonged to one-humped camels [11], followed by the ox and small ruminants [11, 21]. The process of water buffaloes resembled the ox in this respect.

The rostral process arises from the medial and just rostral to the muscular process in small ruminants, whereas the rostral process arises medially form the neck at the same level as at which the muscular process arises in the ox [11], as it was in water buffaloes.

In water buffaloes, the rostral process was attached by a ligament to the wall of tympanic cavity. According to Getty [11], the ossicles in domestic animals are connected with walls of tympanic cavity by ligaments. While in the rodents there is an osseous connection between the process and tympanic cavity [20].

Manubrium. In goats [11], the manubrium is three-sided, whereas the manubrium of water buffaloes was four-sided, like those of ox and sheep [11].

As in the goat and ox [11], in water buffaloes the entire length of manubrium were embedded in PT, whereas in the sheep part of manubrium lies superficially over PT, almost in its middle [11].

The best-developed lateral process belonged to one-humped camels [21], followed by the goat, sheep, and ox [11]. In water buffaloes, the process resembled that of the ox.

In the goat, the lateral process arises almost at the same level as the muscular process [11], whereas in the sheep and ox this process arises caudal to the muscular process, on the opposite side [11], similar to that of water buffaloes.

One of distinguishing features of ossicle morphology among mammals is the orientation of the manubrium relative to the anatomical axis, but this has not been the focus of attention in the larger domestic animals. The manubrium is roughly parallel to the axis in many very small mammals, but it is perpendicular in humans, rabbits, guinea pigs, and chinchillas [20]. In water buffaloes, the manubrium took an intermediate position (not perpendicular or parallel).

Incus. Unlike the ox [35] and water buffalo, the articular surface of body had a concave depression in sheep and goat because of the presence of a high ridge on the surface [11]. The incus body of the ox [35] and water buffalo were a large, well-developed, unlike those of small ruminants [11].

In water buffaloes, the lenticular process was located at the extremity of long crus of incus, as in goats [11], dromedary [21], and Bactrian camels [2]. However, in the sheep and ox the process was absent [11] or present [34, 35].

Stapes. Like the ox [35], this ossicle was nearly rectangular in shape in water buffaloes, whereas it is more trapezoidal in sheep [34].

As in the ox [11], the head of stapes in water buffaloes was convex, whereas the head was flattened in goats [11].

The footplate of mammalian stapes was oval or bean-shaped, as in man. It is elongated, thus having a long and a short axis [10]. As in the ox [35], the shape of footplate was an irregular oval in water buffaloes, whereas it has a squarer shape in sheep [34] and is elliptical in Bactrian camels [2].

CONCLUSIONS

Although general anatomical characteristics of the middle ear structures and the relationship of the ossicles in the water buffalo was almost similar to those in the ox and small ruminates, several distinctive morphologic and morphometric variations of the middle ear structures are recognized in the water buffalo.

In general, among the mammals, there seems to be correlations between ossicle morphology and the frequency that an animal can hear [13, 29]. Therefore, although some of morphological characteristics of middle ear described here remain of unknown functional significance, the middle ear of water buffaloes shows characteristics of the transitional type ear, following the terminology of Fleischer [10]. These characteristics consist of an enlarged malleus head, heavy ossicles, a ligamentous connection of a reduced rostral process to the skull, reduction of malleus anterior lamina, the possession of a relatively large incus, and oblique manubrial orientation to the anatomical axis [20]. Such a transitional type ear leads to the prediction of quite a wide frequency range: not especially low nor especially high. Water buffaloes, like the cattle, belong to the Bovidae family and cattle are able to hear a much wider range of sound frequencies (16 to 40,000 Hz) than humans (20 to 20,000 Hz). This should permit them to hear, in principle, both low-frequency rumbles of elephants and the ultrasonic screams of flying bats [13]. However, intraspecific variations in ossicle morphology among water buffaloes and other ruminants remain to be examined.

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