Mammal Study 34: 7–11 (2009)

Absence of the guttural pouch in a newborn Indian rhinoceros demonstrated by three-dimensional image observations

Hideki Endo[1,*], Hajime Taru[2], Akiko Hayashida[1], Junpei Kimura[3], Takuya Itou[4], Hiroshi Koie[5] and Takeo Sakai[4]

[1] *The University Museum, The University of Tokyo, Tokyo, Japan*
[2] *Kanagawa Prefectural Museum of Natural History, Odawara, Japan*
[3] *Department of Veterinary Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul, Korea*
[4] *Laboratory of Preventive Veterinary Medicine and Animal Health, College of Bioresource Sciences, Nihon University, Kanagawa, Japan*
[5] *Department of Veterinary Internal Medicine, College of Bioresource Sciences, Nihon University, Kanagawa, Japan*

Abstract. CT scanning and its related three-dimensional image techniques were applied for a carcass head of a newborn Indian rhinoceros (*Rhinoceros unicornis*) to clarify if the guttural pouch is well-developed or not in the early growth stages of this species. Observations from the sections of the CT three-dimensional reconstructed image reveal that the guttural pouch is not present around the stylohyoid bone in a new born Indian rhinoceros. Since the absence of the guttural pouch has been confirmed also in adult rhinoceros, we can point out that the guttural pouch does not disappear during the growth stages, but is originally absent in the newborn. Although the well-developed guttural pouch in the horse and ass has attracted anatomists, we can conclude that the guttural pouch is not commonly observed in the perissodactyls, but in a few species of *Equus*, and that the guttural pouch is adapted only to restricted roles in the smaller taxa within perissodactyls.

Key words: CT, growth, guttural pouch, perissodactyls, *Rhinoceros unicornis*.

The accessory apparatus of the respiratory system such as guttural pouch (auditory tube diverticulum) has remained morphologically unclear in large-sized mammals. The perissodactyls have attracted comparative anatomists since the well-developed guttural pouch was observed in the domesticated horse (Bourdelle and Bressou 1949; Way and Lee 1965; Cook 1966; Ellenberger and Baum 1974; von Berg 1974; Sisson 1975; Popesko 1977; König 1984; Dyce et al. 1987; Baptiste and Cake 1994; Budras and Sack 1994; Baptiste et al. 1996), ass (Lindsay and Clayton 1986), and tapir (Turner 1850; Fischer 1986). In contrast we pointed out that among perissodactyls the rhinoceros has no guttural pouch (Endo et al. 1998). Because of these inconsistent results in the perissodactyls, development in the growth of the guttural pouch in the rhinoceroses and its phylogenetic origin in perissodactyls has become noteworthy. In this study, therefore, we applied CT scanning and its related three-dimensional image techniques for the carcass head of a newborn Indian rhinoceros to clarify the possibility of development of the guttural pouch in the early growth stages of the rhinoceroses.

Materials and methods

The head of a newborn Indian rhinoceros (*Rhinoceros unicornis*) of 50 days old that was donated to the Kanagawa Prefectural Museum by Kanazawa Zoological Gardens (Yokohama, Kanagawa, Japan) after pathological checks was used in this study. The head part removed from the cervical vertebrae was axially sectioned by CT (Computed tomography) (Toshiba Aquilion16: Toshiba Medical Systems, Japan) at 0.5 mm thickness without gap. Three-dimensional images were reconstructed from the series of CT sections by Voxel Transmission (Volume Rendering) techniques through an image analyzing system (AZE Virtual Place: AZE Corporation, Tokyo, Japan). Adequate thresholds were

*To whom correspondence should be addressed. E-mail: hendo@um.u-tokyo.ac.jp

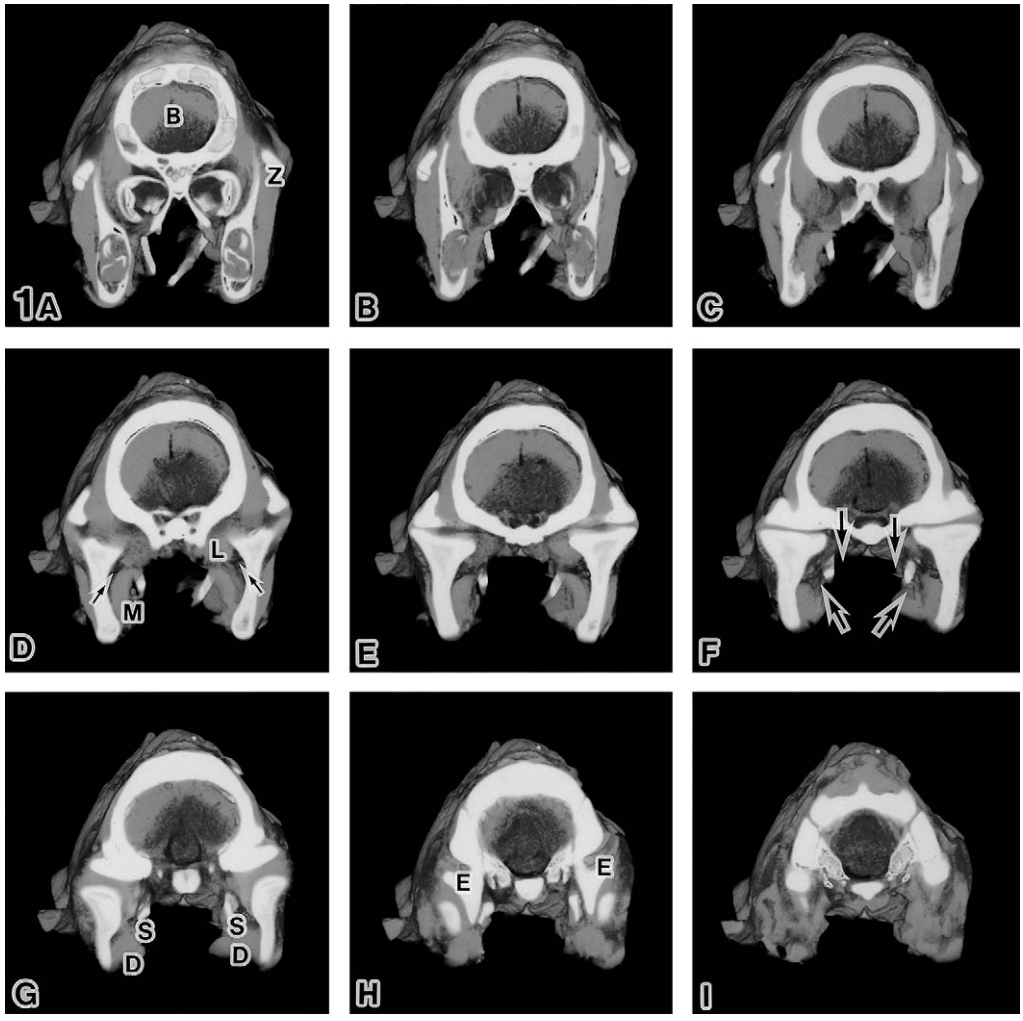

Fig. 1. Axial sections of the three-dimensional reconstructed images of the head of the newborn Indian rhinoceros. The interval between each section is 5 mm from rostral (Fig. 1A) to nuchal plane (Fig. 1L). The thickness of the sections is 0.5 mm. Both sides of the stylohyoid bone (S, Fig. 1G), medial (M, Fig. 1D) and lateral (L, Fig. 1D) pterygoid muscles, digastric muscle (D, Fig. 1G) are shown. The stylohyoid bone consistently accompanies the pterygoid muscles. No vacant space of the guttural pouch is observed around the stylohyoid bone in the entire level (large arrows), although only thin spaces between the bundles of the lateral and medial pterygoid muscles can be seen (small arrows). B (Fig. 1A), brain. Z (Fig. 1A), zygomatic arch. E (Fig. 1H), external auditory meatus.

qualitatively selected and the sections were axially and horizontally obtained from the three-dimensional image to effectively visualize the region of the guttural pouch.

Results

The axial sections of the head from the three dimensional images are shown from a rostral aspect (Fig. 1). The sections are rostro-caudally arranged from the caudal area of the choana to the level of the mandibular articulation and external auditory tube. Since the oral cavity, tongue, pharynx, and larynx were cut and removed for pathological checks, the medial region of the mandibular bodies and the ventral part of the oral cavity were morphologically destroyed. However, the intact areas around the stylohyoid bone could be examined in each section.

The medial pterygoid muscle was observed in rostral section, whereas the lateral pterygoid muscle was seen in caudal space. The stylohyoid bone accompanies the medial pterygoid muscle in ventral space (Fig. 1C and 1D), and is adjacent to the lateral pterygoid muscle in more dorsal area (Fig. 1E and 1F). This indicates that the space corresponding to the guttural pouch is not confirmed in bilateral areas of the stylohyoid bone (Fig. 1A–1G). Although only the thin space is seen in the lateral area of the the bundles of the lateral and medial pterygoid muscles near the stylohyoid bone (Fig. 1D), the

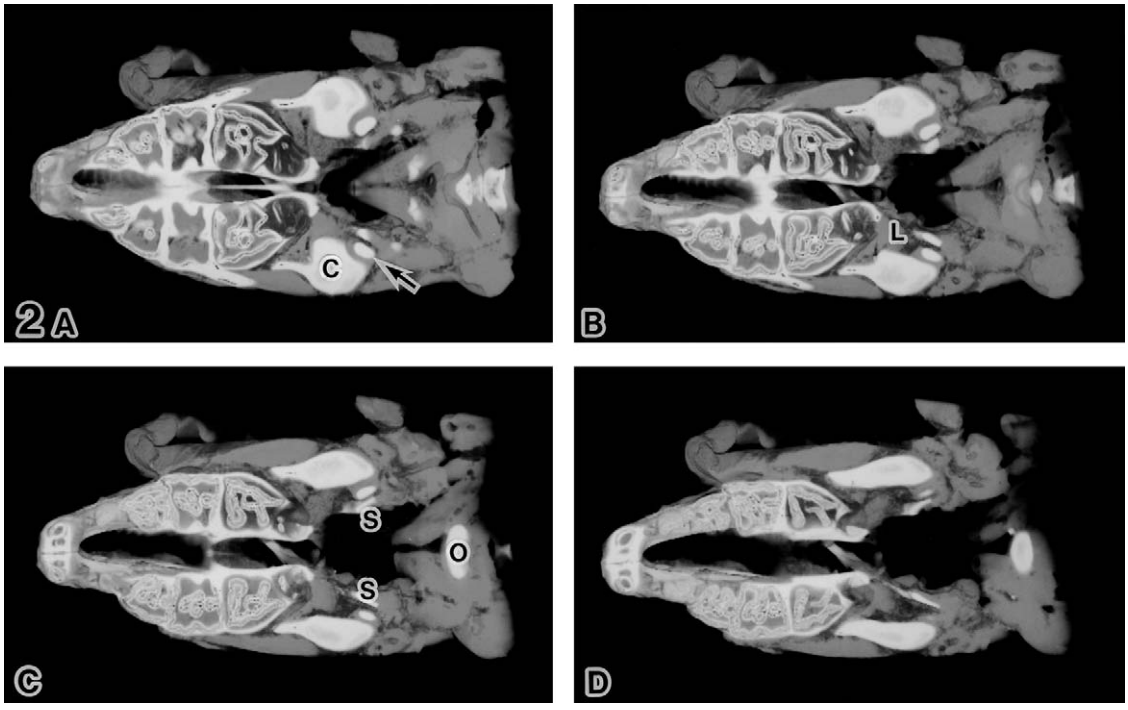

Fig. 2. Horizontal sections of the three-dimensional reconstructed images of the head of the newborn Indian rhinoceros. The interval between each section is 2.5 mm from dorsal (Fig. 2A) to ventral plane (Fig. 2D). The thickness of the sections is 0.5 mm. Both sides of the stylohyoid bone (S, Fig. 2C) and lateral pterygoid muscles (L, Fig. 2B) are shown. The space cannot be observed around the stylohyoid bone. C (Fig. 2A), condylar process of the mandible. Arrow, temporal bone close to the mandibular articulation. O (Fig. 2C), occipital condyle.

auditory tube diverticulum is not present in this region.

The horizontal sections are shown from dorsal to ventral levels (Fig. 2). We can demonstrate that the lateral area of the stylohyoid bone is occupied by the pterygoid muscles (Fig. 2D).

Since the medio-ventral area of the stylohyoid bone was not intact because of the pathological dissection, we could not directly know if the guttural pouch is well-developed or not in this region. However, the space like the auditory diverticulum is not formed in the area close to the pharyngeal recess in the sections of the rostral level (Fig. 1A–1C). In the medio-dorsal area of the caudal level the guttural pouch has been consistently absent (Fig. 1D–1I). Since bundles of lateral pterygoid muscle occupy the medio-rostral space of the mandibular articulation, the guttural pouch cannot take form in the vacant space.

Discussion

The domesticated horse and ass are equipped with a well-developed guttural pouch. The macroscopic structure of the guttural pouch has been examined in detail by X-ray (Trigo and Nickls 1981; Bayly and Robertson 1982; Freeman 1991; Hance et al. 1992; Sweeny et al. 1993) and CT observations (Sasaki et al. 1999) and the mold specimen approach (König 1984; Baptiste et al. 1996; Manglai et al. 2000a).

The pouch is symmetrical and the formation of the thin septum is confirmed in the sagittal plane (Dyce et al. 1987; Baptiste et al. 1996; Sasaki et al. 1999). The volume of the guttural pouch accounts for 4.3% of the entire head (Kubo et al. 1992) and reaches 472 cm^3 in cases of the domesticated horse (Sisson 1975), while the size and shape of the guttural pouch is changeable according to the contraction and relaxation of the muscles surrounding the pouch (Baptiste et al. 1996; Baptiste 1998). As shown in the horse, the typical guttural pouch is divided into two parts, the lateral and medial compartments, at each side of the stylohyoid bone (Popesko 1977; Sasaki et al. 1999). So, in this study, the two compartments were studied using the stylohyoid bone as a landmark. However, neither lateral nor medial chambers were not observed around the stylohyoid bone.

The ventro-caudal area of the medial compartment is well-developed in the domesticated horse (Dyce et al. 1987; Baptiste and Cake 1994; Baptiste et al. 1996) in contrast to its much smaller capacity in the Przewalski's

horse (Sasaki et al. 1999). From the comparative data between the Przewalski's and domesticated horses, we suggest that the ventro-caudal part of the medial compartment of various horse breeds has varied in both volume and shape through the domestication process based upon the requirements of higher respiratory function. For example, the relative size of the guttural pouch has been artificially selected and become enlarged during the domestication of the various breeds as transporting and racing animals. Since the present image data on the medial region is reconstructed from a partially-destroyed head, detailed findings should be obtained from intact specimens in the future. However, we can conclude that any vacant space such as a guttural pouch cannot be formed even in the medial region.

The absence of the guttural pouch was demonstrated in macroscopic observations of the white rhinoceros (Endo et al. 1998). Since the guttural pouch is not present in the newborn Indian rhinoceros in the present study, we can point out the guttural pouch does not disappear during growth stages, but is originally absent around the stylohyoid bone. We can conclude that the guttural pouch is not commonly observed in perissodactyls. We can point out that the guttural pouch has been confirmed rather in only a few species of horses and tapirs, since the guttural pouch occurs in ass (Lindsay and Clayton 1986) and tapir (Turner 1850; Fischer 1986).

It has been suggested that the guttural pouch may play a role in the exchange of air as a part of the respiratory organ (Fish 1910; Dyce et al. 1987; Baptiste and Cake 1994). Other reports have stated that the function of the guttural pouch may be to help the pharynx to swallow food (Skoda 1911; Rooney 1997), or that the guttural pouch may act as an air ventilator to cool the blood flow of the internal carotid artery, at least in the domesticated horse (Baptiste et al. 1993; Baptiste 1997, 1998). It was pointed out that the mechanism may contribute to brain-cooling using the blood flow of the internal carotid arteries closely-positioned in the guttural pouch of race horses during heavy exercise (Baptiste et al. 2000). Yet another report suggested that the guttural pouch may function as an immunological organ in the respiratory system of horses (Manglai 2000b).

Since the data have demonstrated that the structure of the guttural pouch has been morphologically established only in a few taxonomic groups, we suggest that the guttural pouch may be actually adapted only to restricted roles in the smaller taxa within perissodactyls. We think that the functional significance of the guttural pouch may be generally unimportant in mammals except for *Equus*, even if in the future the smaller space related to the Eustachian tube is to be found in species other than the horse, ass, and tapir.

Acknowledgments: We wish to express our gratitude to Kanazawa Zoological Gardens for the donation of a rare carcass of the Indian rhinoceros. This study was supported by Grant-in-Aids for Scientific Research nos. 17405018, 18637001 and 19405001 from the Ministry of Education, Science and Culture, Japan and by JSPS core-to-core program HOPE.

References

Baptiste, K. E. 1997. Functional anatomy observations of the pharyngeal orifice of the equine guttural pouch. (auditory tube diverticulum). Veterinary Journal 153: 311–319.

Baptiste, K. E. 1998. A preliminary study on the role of the equine guttural pouches in selective brain cooling. Veterinary Journal 155: 139–148.

Baptiste, K. E., Grandage, J. and Johnson, K. 1993. Is the guttural pouch of the horse a brain-cooling device? Journal of Anatomy 183: 174.

Baptiste, K. E. and Cake, M. H. 1994. Lipid analysis of lavage samples from the equine guttural pouch (auditory tube diverticulum). Annals of Otology, Rhinology & Laryngology 103: 383–388.

Baptiste, K. E., Holladay, S. D. and Freeman, L. E. 1996. Alterations in equine guttural pouch morphology with head position: observations using a new technique for producing accurate casts. Anatomical Record 246: 579–584.

Baptiste, K. E., Naylor, J. M., Bailey, J., Barber, E. M., Post, K. and Thornhill. J. 2000. A function of guttural pouch in the horse. Nature (London) 403: 382–383.

Bayly, W. M. and Robertson, J. T. 1982. Epistaxis caused by foreign body penetration of a guttural pouch. Journal of American Veterinary Medical Association 180: 1232–1234.

Bourdelle, E. and Bressou, C. 1949. I. Equidés. In (E. Bourdelle and C. Bressou, eds.) Anatomie Régionale des Animaux Domestiques. Livre V, pp. 315–334. Baillière, Paris.

Budras, K. D. and Sack, W. O. 1994. Anatomy of the Horse. An Illustrated Text. 2nd ed. Mosby-Wolfe, London, pp. 135.

Cook, W. R. 1966. Clinical observations on the anatomy and physiology of the equine upper respiratory tract. Veterinary Record 79: 440–446.

Dyce, K. M., Sack, O. W. and Wensing, C. J. G. 1987. Textbook of Veterinary Anatomy. W. B. Saunders, Philadelphia, pp. 820.

Ellenberger, W. and Baum, H. 1974. Handbuch der Vergleichenden Anatomie der Haustiere. 18th ed. Springer, Berlin, pp. 1155.

Endo, H., Manglai, Fujisawa, M., Kurohmaru, M. and Hayashi, Y. 1998. The guttural pouch is not present in the white rhinoceros (*Ceratotherium simum*); Morphology of the Eustachian tube and nasopharynx. Anatomia, Histologia and Embryologia 27: 327–330.

Fischer, M. S. 1986. Die Stellung der Schliear (Hyracoidea) im phylogenetischen System der Eutheria. Courier Forschungsinstitut Senckenberg 84: 1–132.

Fish, P. A. 1910. The exchange of air in the eustachian or guttural pouches of the horse. American Journal of Physiology 27: 229–232.

Freeman, D. E. 1991. Equine Respiratory Disorders. Lea & Febiger, Philadelphia, pp. 458.

Hance, S. R., Robertson, J. T. and Bukowiecki, C. F. 1992. Cystic structures in the guttural pouch (auditory tube diverticulum) of two horses. Journal of American Veterinary Medical Association 200: 1981–1983.

König, H. E. 1984. Zur Topographie des Luftsackes (Diverticulum tubae auditivae) beim Pferd. Tierarztliche Praxis 12: 219–227.

Kubo, K., Sakai, T., Sakuraoka, H. and Ishii, K. 1992. Segmental body weight, volume and mass genter in thoroughbred horses. Japanese Journal of Equine Science 3: 149–155.

Lindsay, F. E. and Clayton, H. M. 1986. An anatomical and endoscopic study of the nasopharynx and larynx of the donkey (*Equus asinus*). Journal of Anatomy 144: 122–132.

Manglai, D., Wada, R., Endo, H., Kurohmaru, M., Yoshihara, T., Sasaki, M. and Hayashi, Y. 2000a. Macroscopic anatomy of the auditory tube diverticulum (guttural pouch) in the throughbred equine. A silicone mold approach. Okajimas Folia Anatomica Japonica 76: 335–346.

Manglai, D., Wada, R., Kurohmaru, M., Sugiura, T., Yoshihara, T., Oikawa, M. and Hayashi, H. 2000b. Distribution of immunoglobulin isotypes and subisotypes in equine guttural pouch (auditory tube diverticulum). Journal of Veterinary Medical Science 62: 1001–1003.

Popesko, P. 1977. Atlas of Topographical Anatomy of the Domestic Animals. Vol. 1, 2nd ed. W. B. Saunders, Philadelphia, pp. 619.

Rooney, J. R. 1997. Why guttural pouches? The Equine Disease Quarterly 6: 3.

Sasaki, M., Hayashi, Y., Koie, H., Yamaya, Y., Kimura, Y., Manglai, D., Kawashima, S., Endo, H. and Yamamoto, M. 1999. CT examination of the guttural pouch (auditory tube diverticulum) in Przewalski's horse (*Equus przewalskii*). Journal of Veterinary Medical Science 61: 1019–1022.

Sisson S. 1975. The ear. In (R. Getty, ed.) Sisson and Grossman's the Anatomy of the Domestic Animals Vol. 1, 5th ed, pp. 719–727. W. B. Saunders, Philadelphia.

Skoda, K. 1911. Uber die Bedeutung der Tubendivertikel (Luftsacke) bei den Equiden. Anatomische Hefte. Abteilung 1. 42: H3.

Sweeny, C. R., Freeman, D. E., Sweeney, R. W., Rubin, J. L. and Maxson, A. D. 1993. Hemorrhage into the guttural pouch (auditory tube diverticulum) associated with rupture of the *longus capitis* muscle in three horses. Journal of American Veterinary Medical Association 202: 1129–1131.

Trigo, F. J. and Nickls, F. A. 1981. Squamous cell carcinoma of a horse's guttural pouch. Modern Veterinary Practice 62: 456–459.

Turner, H. N. 1850. Contributions to the anatomy of the tapir. Proceedings of the Zoological Society of London 1850: 102–105.

von Berg, R. 1974. Angewandte und Topographische Anatomie der Haustiere. Ferdinadenke, Stuttgart, pp. 416.

Way, R. F. and Lee, D. G. 1965. The Anatomy of the Horse. Lippincott, Philadelphia, pp. 214.

Received 3 October 2007. Accepted 14 August 2008.