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EXPLORING A DISSECTION-BASED GROSS ANATOMY OF CRANIAL NERVE ROOTS IN WATER BUFFALO (*Bubalus bubalis*)

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ABSTRACT

Understanding the cranial nerve anatomy is essential for accurate neurological examination, and provides a framework for performing nerve blocks during surgical procedures. However, there is scarcity of information on the gross anatomy of cranial nerves in water buffalo, and this study was aimed to comprehensively explore the gross anatomy of cranial nerve roots in water buffalo brain. Forty adult buffalo heads were procured from local slaughter houses and their cranial nerve roots exposed using the ventral and lateral approaches. The findings revealed that the gross anatomy of buffalo cranial nerve roots closely resemble that of other ruminants. The intracranial course of cranial nerves innervating the orbital region was influenced by the location of their origin within the brain and their entry points into the cranial dura mater. Unlike vestibulocochlear and facial nerve, which appeared as solid cylindrical structures, the vagus group and hypoglossal nerves comprised of multiple rootlets. The hypoglossal nerve, in addition to its predominant root, presented a small root emerging out through an accessory foramen. Consistent with other ruminants, the vagus group originated from dorsolateral aspect of medulla and traversed through jugular foramen, cranial to the hypoglossal foramen. Based on this study the first five cranial nerves emerging from the brain stem are best visualised using ventrolateral approach, while the remaining cranial nerves are more effectively observed through ventral approach.

Keywords: Buffalo, cranial nerve roots, dissection, gross anatomy, manual approach

INTRODUCTION

The cranial nerves form an important component of the peripheral nervous system as it is associated with the ganglia-like cranial cervical ganglion, otic ganglion and Gasserian ganglion (Najafi *et al*., 2011; Nourinezhad *et al*., 2016). Except the olfactory nerve, the cranial nerves are associated with the brain stem. The nerves have a short intracranial course before reaching their target structures which include eye, ear, face, larynx, and the other vital components of head and neck region. Facial nerve paralysis, laryngeal hemiplegia, rabies and dysphagia are frequently encountered clinical conditions which demand a basic understanding of the cranial nerve anatomy (De Lahunta and Habel, 1986). A detailed report is available in text books on the gross anatomy of the cranial nerves in domestic animals (Singh, 2017). Buffalo contribute to more than 50% of the meat and milk production in South Asia (Banerjee, 1998). To our best knowledge, although a few reports are available on the anatomy of cranial nerves in buffaloes (El-Shaieb *et al*., 1990; Sunil Kumar *et al*., 2018, 2019), a comprehensive study on the entire cranial nerve roots in this species is lacking. Secondly, there is a lack of clear photographic evidence regarding the gross anatomy of cranial nerves in this species. Keeping all these factors in mind, the present research is intended to analyse the gross anatomy of cranial nerve roots in buffalo brain.

MATERIALS AND METHODS

Collection of samples

Forty adult water buffalo (*Bubalus bubalis* L.) heads, independent of sex, and collected from local slaughter houses in Srinagar, Jammu & Kashmir (India), were immediately fixed in 10% formalin for two days. The approximate age of animals, as determined by dentition (Banerjee, 1998), ranged between 6-8 years. Considering that the buffalo animals were not euthanized or hunted for experimental purposes, and heads were procured from the slaughter houses. The ethical committee approval was not required for this study.

Gross anatomy

To expose the cranial nerve roots, using osteotome, 40 cranial cavities were exposed through the ventral approach (John *et al*., 2024). The basioccipital, together with the body of sphenoid bone, was wedged out with the help of periosteal elevator and the meningeal cover was cut along the mid axis of the brain stem. In 13 buffalo skulls, the wings and the pterygoid processes of sphenoid bone were cut with the help of the bone cutter. The remaining seven cranial cavities were exposed using the dorsal approach (Ashwini *et al.*, 2008). The dura mater was cleared from the cranial periosteum using blind dissection, similar to the approach described earlier in mammals (Deepthi *et al*., 2016). The dura mater was then incised at multiple locations to allow proper fixation in 10% formalin in which the samples were kept immersed for 3 weeks (John *et al*., 2024). The gross anatomy of cranial nerve roots was observed macroscopically and photographs of cranial nerves were captured using iPhone 13 camera (12 MP, Apple).

RESULTS AND DISCUSSION

The current study documents the first comprehensive report about the overview of the gross anatomy of cranial nerve roots in water buffalo. The results revealed that the pattern of cranial nerve root in buffalo resembled large ruminants in general. In cranial cavity, the optic nerve was the dorsal most nerve related to the brain stem. Observed just behind the rostral cerebral artery (John *et al*., 2024), the nerve coursed through the optic canal (Fig. 1). The oculomotor nerve emerged as a flat nerve from the ventral aspect of cerebral crura in mid brain. Coursing rostrally the nerve then pierced the dura mater just lateral to the emergent artery (Fig. 1 & 2). The oculomotor nerve then coursed rostrally, and covered by the cavernous

Fig. 1: Ventrolateral view of the buffalo brain showing optic nerve (2), oculomotor nerve (3), trochlear nerve (4), Rostral cerebral artery (RCA), Emergent artery (EA), Mesencephalic tectal branches (MTB) and RCbA (Rostral cerebellar artery)

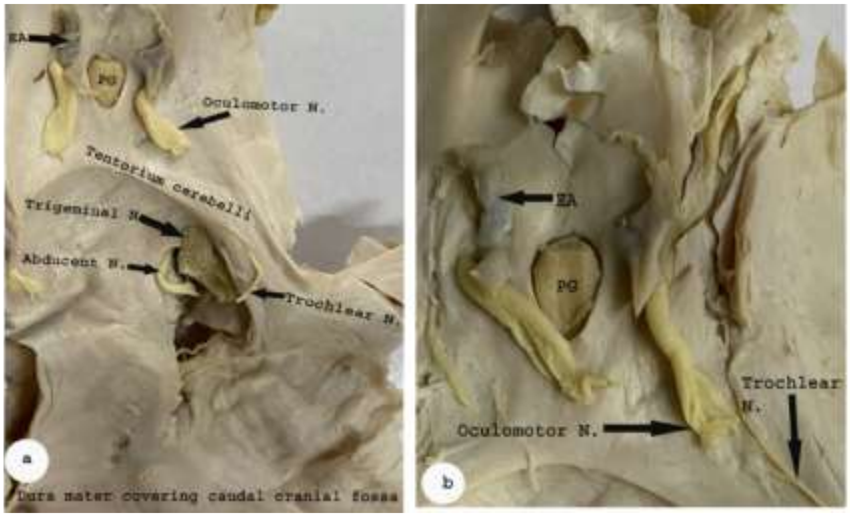

Fig. 2: Dura mater showing the piercing area of the oculomotor nerve around the pituitary gland (PG), lateral to the emergent artery (EA) and the trigeminal nerve; the trochlear and the abducent nerve piercing the dura mater dorsolateral and ventrolateral to the trigeminal nerve, respectively.

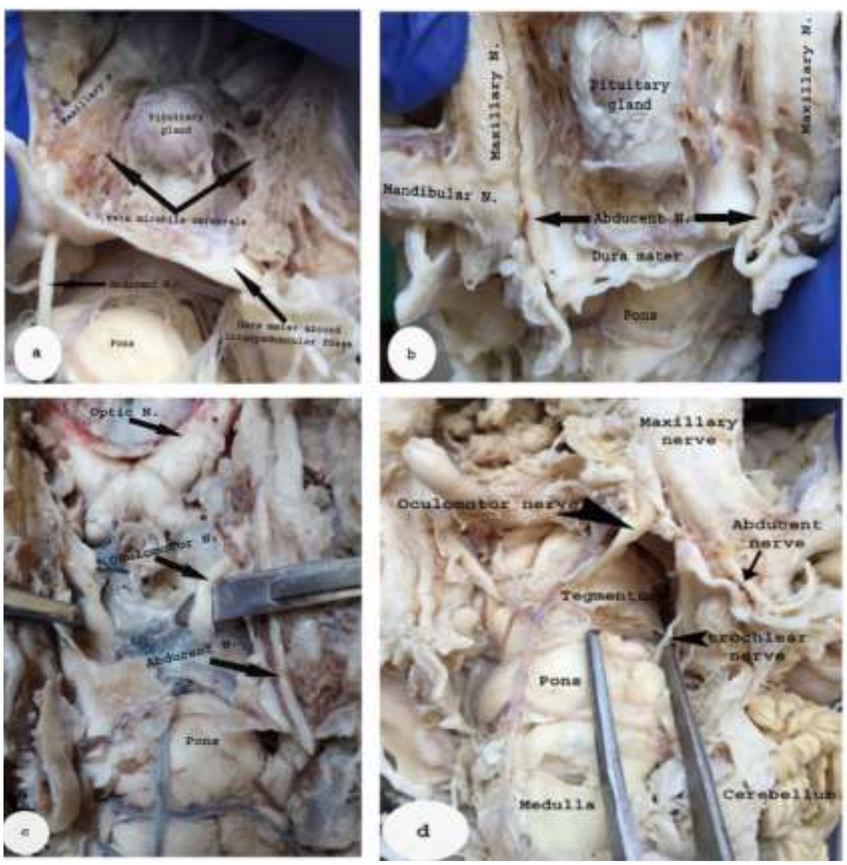

Fig. 3: Ventral view of the buffalo brain showing the interpeduncular region and the associated nerves

sinus on the ventral aspect of the brain, it was related to the medial aspect of maxillary branch of trigeminal nerve (Fig. 2 & 3). Our findings are in conformity with deLahunta *et al.* (2021). Contrary to our observation of oculomotor nerve being a large single root in water buffalo, El-Shaieb *et al*. (1990) reported multiple rootlets in oculomotor nerve in Egyptian buffalo (*B. bubalis).* The trochlear nerve, which appeared as a fine thread (Fig. 3), and was closely associated with the rostral cerebellar artery (John *et al*., 2024), emerged from the dorsal aspect of brain stem just caudal to the caudal colliculi (Fig. 1). The nerve crossed laterally and ventrally in front of pons and pierced the tentorium cerebelli dorso-lateral to trigeminal nerve (Fig. 2 & 3). Just lateral to the course of oculomotor nerve, the nerve coursed along dorsolateral aspect of ophthalmic branch of the trigeminal nerve. trigeminal nerve was largest among all the cranial nerves. The trochlear and trigeminal nerves reportedly are the finest and largest among all cranial nerves, respectively, in domestic animals (Singh, 2017). After originating from the caudolateral aspect of pons in brain stem, trigeminal nerve pierced the dura mater just ventral to the tentorium cerebelli (Fig. 2). The ventral and dorsolateral view deciphered the nerve to comprise of a larger and more cylindrical root, comprising of the smaller dorsal ophthalmic branch with a larger ventral maxillary branch, and a relatively smaller root formed of mandibular branch (Fig. 3 & 4). The two roots were placed at an acute angle to each other, with the former root exiting through foramen orbitorotundum and the mandibular part emerging via foramen oval (Fig. 4). The sensory root of trigeminal nerve appeared a cylindrical structure related to the trochlear and oculomotor nerve at its dorsolateral and dorsomedial aspect, respectively. The gross anatomy of the trigeminal nerve in all water buffaloes was somewhat similar to that of cattle (Getty, 1975). At the craniolateral aspect of pyramids, the abducent nerve roots emerged from the ventral aspect of brainstem (Fig. 5 & 6). At its origin, the nerve appeared to have multiple roots (3-4) before acquiring a cylindrical shape. The nerve travelled an appreciable distance and pierced the dura mater medial to the maxillary branch of trigeminal nerve (Fig. 3). Ventrally, in the area of cavernous sinus, the nerve was related to the rostral branch of epidural rete mirabile, whereas laterally it was in close association with the maxillary nerve (Fig. 3). These findings are in agreement with Levine *et al.* (2008) in case of ruminants. The intracranial course of cranial nerves associated with the eye ball and its adnexa (2nd, 3rd, 4th, 5th and 6th cranial nerves) resembled to cattle in general (Getty, 1975). The overall arrangement of optic, oculomotor, trochlear, trigeminal, and abducent cranial nerves in buffaloes was observed as: the trigeminal nerve being in the intermediate position; the oculomotor and the trochlear nerve positioned at dorsomedial and dorsolateral locations, respectively, with respect to the sensory root of trigeminal; and the abducent nerve located at the ventromedial aspect of trigeminal component. This arrangement of cranial nerves in buffalo, also reported in cattle (Singh, 2017), should therefore be taken into consideration, since it defines the principles of anesthetising these cranial nerves while

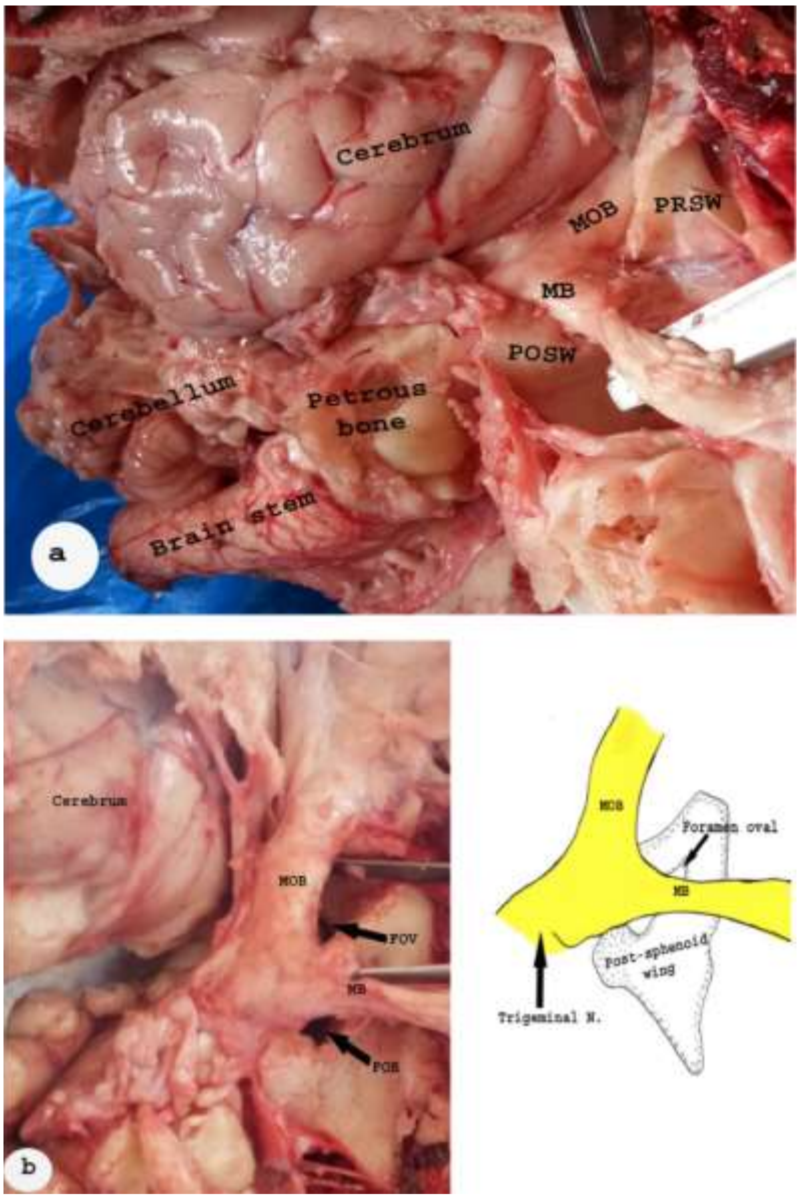

Fig. 4: Dorsolateral view of water buffalo brain (4a, b) showing maxillary branch (MB), maxilla-ophthalmic branch, postsphenoid wing (POSW), presphenoid wing (PRSW), foramen oval (FOV) & foramen orbito-rotundum (FOR)

using the retro-bulbar technique and Peterson's nerve block (Mansour *et al*., 2018). Just behind the pons and released from the dorsolateral aspect of the medulla, the facial and vestibulocochlear nerves were placed adjacent to each other and entered into the internal auditory meatus of petrous bones (Fig. 5 & 6). Both these nerves appeared cylindrical in shape. The vestibule-cochlear nerve was differentiated from the facial by being caudal and in the inferior fossa of internal acoustic meatus. The facial nerve was located in the superior fossa of petrous temporal. The glosso-pharyngeal, vagus and accessory spinal nerves were given off from the lateral aspect of medulla oblongata about an inch caudal to the origin of facial and vestibulocochlear nerve (Fig. 5 & 6). The intracranial course of vagus and glossopharyngeal nerves was closely associated with each other to the extent that they were nearly inseparable. This could be attributed to the fact that these two nerves innervate, in common, the structures of pharyngeal region (Levine *et al*., 2008), beyond which the vagus nerve takes a separate course to innervate the more distal structures of the body. The accessory spinal nerve was formed of a distinct spinal part and a cranial part, as confirmed in Goitred Gazelle and Tuj Sheep (Aslan *et al*., 2006). The spinal part was almost double the size of the cranial part (Fig. 6). The larger size of spinal branch may be explained for it being the main nerve supply to the heavy neck musculature (Levine *et al*., 2008). There are contrary reports, though, in

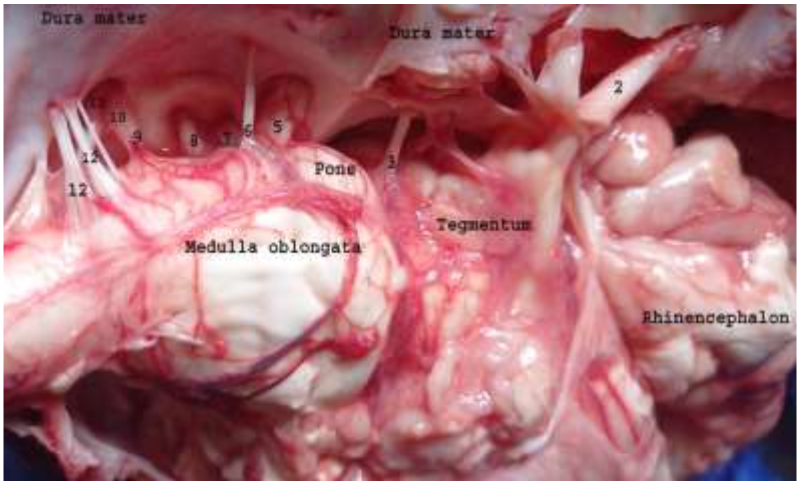

Fig. 5: Ventrolateral view of water buffalo brain showing the cranial nerves emerging from the brain stem in sequential order; 2 - optic 3 -oculomotor (lateral to emergent artery-EA), 5 - trigeminal, 6 - abducent, 7 - facial, 8 - vestibulocochlear, 9 - glossopharyngeal, 10 - vagus, 11 - accessory spinal and 12 - hypoglossal nerve.

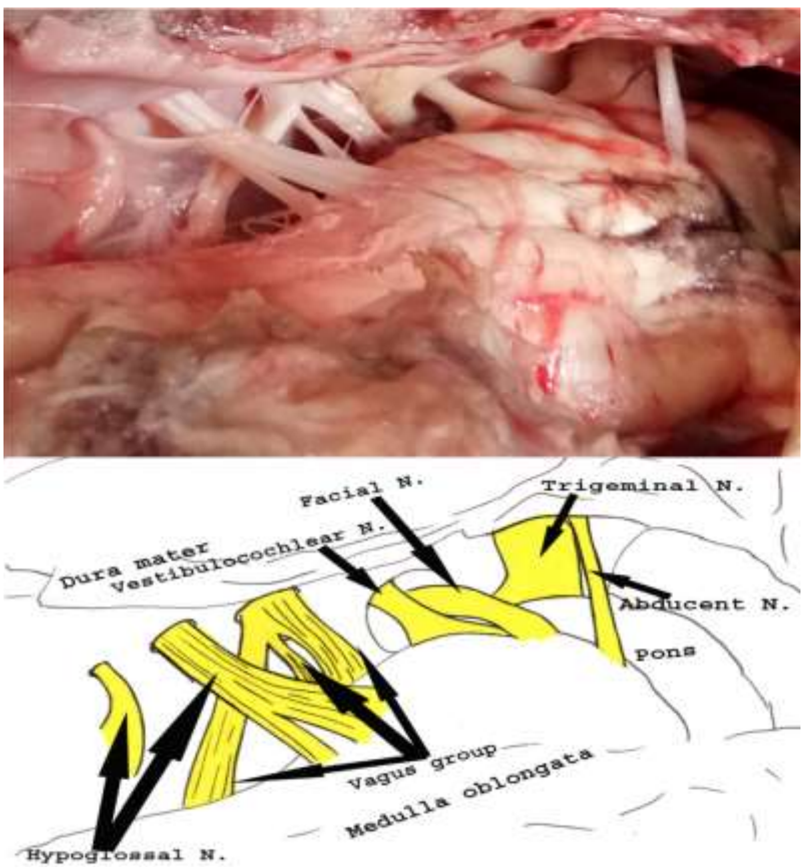

Fig. 6: Ventrolateral view of the medulla oblongata of water buffalo brain and the associated cranial nerves

animals like camel, giraffe, and lama, where the spinal root is not present at all (Cui-Sheng and Xie, 1998). The accessory nerve, along with the vagus and glossopharyngeal nerves emerged outside the cranial cavity through the jugular foramen. This is in contrast to the earlier reports (Rao and Rao, 1975), where the nerve reportedly emerged through foramen lacerum. Hypoglossal nerve was located at the caudolateral aspect of pyramids (Rao and Sharma, 1974), and featured multiple rootlets, ranging from 8 to 12 (Fig. 6), which fused just before their emergence through the hypoglossal foramen (Fig 6). In 20% of the cases, additional rootlets of this nerve exited out through an accessory foramen. The multiple rootlets of nerve have also been observed in the human and elephant brain (Shoshani *et al*., 2006).

To our best information, the methods pertaining to the exposure of the cranial nerve roots in animals lacks comprehensive details in general (Aslan *et al*., 2006; Bahar *et al*., 2006), and buffalo in particular (Rao and Sharma, 1974; Rao and Rao, 1975). Considering that the cranial nerve roots are closely associated with the rete mirabile cerebral and the arterial circle of brain in ruminants, including buffalo (John *et al*., 2024), some critical points need addressal, the optic nerve could be best explored by targeting the pre-sphenoid wing from the rostro-lateral aspect of the cranial cavity; the course of oculomotor and the abducent nerves are in close proximity to rete mirabilis cerebrale in the region of interpeduncular fossa, suggesting these nerves to be explored via the ventral approach, not without teasing the rostral rete; lastly, the large sized trigeminal nerve and the ventrally bulged pons hamper the visualisation of the nerves of the orbital region through the ventral approach. To avoid this complication, the cranial cavity should be approached laterally (Godinho, 1968) and explored at the level of foramen oval and the oribitorotundum, which are demarcated from each other by the post-sphenoid wing. The last seven cranial nerves (6-12) emerge from the medulla region resting on the basioccipital bone which, together with the occipital condyle should therefore be wedged out.

Conclusion: The study revealed that the gross anatomy of cranial nerve roots in general resembled with other ruminants. The first five cranial nerves emerging from the brain stem were best visualised using ventrolateral approach. The remaining cranial nerves were best observed using ventral approach. The findings may help in the better execution of different anaesthetic techniques to block the cranial nerves, in particular the nerves innervating the orbital region.

Consent for publication: All authors declare to have conflict of interest regarding this paper.

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