

GROSS AND HISTO-ARCHITECTURAL STUDIES ON THE NERVOUS TUNIC OF THE EYEBALL OF MURRAH BUFFALO

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SUMMARY

The nervous tunic of the eyeball is responsible for proper vision of the animal. The retina changes the light impulse into electrical signals. These signals are then transmitted via the optic nerve, to the occipital cortex of the brain. Here, the brain interprets the electrical signals into visual images. In the present finding, the retina was observed to be a delicate innermost tunic of the eyeball extending from the optic nerve's entrance to the pupil's margin. Microscopically, the sensory part of the retina was composed of ten layers. The average thickness of the retina was $144.75 \pm 1.61 \mu\text{m}$. The micrometrical observations revealed that the thickness of the various retinal layers in Murrah buffalo was less than that of bovines and also varied within the breed.

Keywords: Buffalo, Eye ball, Histology, Nervous tunic, Retina

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The vision is the most essential sense. For many animals, light perception is the trigger and the controlling reason for behaviors such as "seeing and doing". Many biological processes display circadian rhythms in activity, which presumably operate to coordinate cellular function with daily environmental oscillations. Many ocular tissues exhibit circadian rhythms to optimize specific processes requiring coordination with the light-dark cycle. The retina conveys the diurnal changes in environmental illumination to the brain to entrain circadian rhythms throughout the body. Within the layers of the retina, light impulses are changed into electrical signals. These signals are then transmitted via the optic nerve, to the occipital cortex of the brain. Here, the brain interprets the electrical signals into visual images (Dalga *et al.*, 2022). Looking at the importance of this nervous tunic and the paucity of literature on buffaloes, this work was designed to explore the gross and histological aspects of the nervous tunic of the eyeball of Murrah buffalo.

Twenty pairs of eyeballs from adult physically healthy Murrah buffaloes (*Bubalus bubalis*) of both sexes were collected from the Tangra slaughter house, Kolkata, a government-authorized abattoir of West Bengal. The samples were preserved in 10% neutral buffered formalin and Davidson's fixative. After measuring the gross parameters, the samples were subjected to standard tissue processing and sectioning procedure. The sections were stained with Haematoxylin and Eosin (H&E) for histological observations (Luna, 1968).

H &E-stained slides were used for micrometry by Leica Qwin Images Analyzer software in Leica DM 2000 Microscope.

The retina was observed to be a delicate innermost tunic extending from the optic nerve's entrance to the pupil's margin. Its inner surface was in contact with the hyaloid membrane of the vitreous humor, and its outer surface was in connection with the choroid coat. Similar observations had been reported by Soliman *et al.* (2010) in goat. The retina was divided into three concentric zones: (1) the pars optica retinae, the most significant posterior part. (2) the pars ciliaris retinae that continued over the ciliary body in the form of two layers. The inner layer was non-pigmented while the outer layer was the direct continuation of the pigment layer of the pars optica. (3) the pars iridica retinae that extended on the posterior surface of the iris, in the form of a layer of pigmented cells. The present observation was in agreement with the reports of Kima *et al.* (2018) in Surti buffaloes and Rajathi and Muthukrishnan (2022) in sheep and goat.

Microscopically, retina was composed of a sensory and a non-sensory portion. The sensory portion was referred to as the pars optica retinae that comprised of ten layers which were from outside to inside as follows: (1) Pigmented epithelium (PE) layer (2) Layer of rods and cones (3) Outer limiting layer (4) Outer nuclear layer (5) Outer plexiform layer (6) Inner nuclear layer (7) Inner plexiform layer (8) Ganglion cell layer (9) Outer nerve fiber layer and (10) Internal limiting membrane (Fig.1). Similar observations were reported by Kima *et al.* (2018) and Babu *et al.* (2022) in buffaloes, Dalga *et al.* (2022) in sheep and Rajathi and Muthukrishnan (2022) in sheep and goat. The average thickness of the retina was $144.75 \pm 1.61 \mu\text{m}$; however, it was reported to be $177.56 \pm 10.72 \mu\text{m}$ thick by Kima *et al.* (2018) in Surti buffaloes that might be a breed variation within the species. The retinal pigmented

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layer comprised of simple squamous or cuboidal epithelium resting on a basal lamina. The basal part of the cells included a spherical nucleus lying peripherally to the basal lamina with several round granules of melanin. The epithelial cells were pigmented except in the area where the tapetum fibrosum was present. The present finding was in accordance with the reports of Kima *et al.* (2018) in Surti buffaloes, Soliman *et al.* (2010) in goat and Rajathi and Muthukrishnan (2022) in sheep and goat. The basement membrane of the epithelium and the endothelium of the chorio-capillaris layer formed a basal complex known as Bruch's membrane (Fig. 1) which was in accordance with the reports of Babu *et al.* (2022) in buffaloes. The thickness of the pigmented epithelium layer was 3.81 ± 0.24 μm which was lower than the micrometrical observations reported by Kima *et al.* (2018) in Surti buffaloes and Babu *et al.* (2022) in buffaloes.

The next layer of Rod and Cone cells consisted of an outer segment, which was a photo sensitive part, and an inner segment which included the nucleus and cytoplasm. The outer segmented layer was adjacent to the pigmented epithelium and the inner segmented layer blended just after the outer segmented layer. These two layers comprised of rods and cones cell respectively which was in accordance with the findings of Kima *et al.* (2018) and Babu *et al.* (2022) in buffaloes and Soliman *et al.* (2010) in goats. The inner cone segment was found to contain the nucleus that was larger. The nuclei of the cones were arranged in a single row immediately beneath the outer limiting membrane (Fig. 1). The layer of the Rod and Cone cells thickness was 15.52 ± 0.98 μm . Khaled (2003) in bovines, Kima *et al.* (2018) in Surti buffaloes and Babu *et al.* (2022) in buffaloes also reported a higher value than the present observation.

The next layer was the outer limiting layer. It separated the inner limiting membrane and the outer nuclear layer (Fig. 1). This membrane contained elastic fibers. The thickness of the outer limiting layer was 0.74 ± 0.03 μm . The present observation was not in accordance with the findings of Khaled (2003) in bovine.

The outer nuclear layer was composed mainly of nuclei of rods and cones arranged in 5-7 rows (Fig. 2) in contrary to the findings of Kima *et al.* (2018) who reported it to be arranged in 7-9 rows, but similar to the findings of Soliman *et al.* (2010) who reported it to be of 6-7 rows. The cone cells nuclei were located in the proximity of this layer and formed only a single row. In contrast, the nucleus of the rods formed several layers in the inner portion of this layer. The outer nuclear layer thickness was 23.14 ± 0.25 μm . The present observation was lower than that of the findings of Khaled (2003) in bovine and Kima *et al.* (2018)

in Surti buffalo.

The outer plexiform layer was a thin layer that separated the outer nuclear layer from the inner nuclear layer (Fig. 1) which was in accordance with the reports of Kima *et al.* (2018) in Surti buffalo. It was composed mainly of the horizontal cell processes. Similar observation was reported by Babu *et al.* (2022) in buffaloes and Rajathi and Muthukrishnan (2022) in sheep and goat. The thickness of the outer plexiform layer was 8.03 ± 0.41 μm which was slightly higher than the reports of Kima *et al.* (2018) in Surti buffalo but lower than the reports of Babu *et al.* (2022) in buffaloes and Khaled (2003) in bovines.

The next layer was inner nuclear layer. This layer separated the outer and the inner plexiform layers. Its nuclei are arranged mostly in 3 rows (Fig. 2) that was similar to the findings of Kima *et al.* (2018) and Soliman *et al.* (2010). It was composed mainly of four cell types: horizontal, bipolar, amacrine and supporting glia (Müller's) cells. The horizontal cells had large and lightly stained nuclei with prominent single nucleolus. It was located along the outer margin of the inner nuclear layer. The amacrine cell nuclei were located vertically in the inner nuclear layer and were euchromatic. The nuclei of the bipolar cells and Muller's cells were situated in the center zone of the inner nuclear layer. Nuclei of the Muller's cell were angulated and had denser chromatin. Bipolar cells formed the largest population in this layer and were characterized by euchromatic to heterochromatic nuclei. Similar observations were also reported by Babu *et al.* (2022) in buffaloes and Rajathi and Muthukrishnan (2022) in sheep and goat. The thickness of the inner nuclear layer was 18.16 ± 0.74 μm that was lower than the reports of Kima *et al.* (2018) and Babu *et al.* (2022) in buffaloes but was almost double than the findings of Khaled (2003) in bovines.

The inner plexiform layer comprised of bipolar and amacrine cell axons (Fig. 1) which was in accordance with the reports of Babu *et al.* (2022). The thickness of this layer was 46.25 ± 0.92 μm . The present observation was in accordance with the reports of Babu *et al.* (2022) in buffaloes but higher than that of the findings of Khaled (2003) in bovine and Kima *et al.* (2018) in Surti buffaloes.

The ganglion cell layer comprised of the retinal ganglion cells nuclei and cell bodies arranged in one or several layers (Fig. 1). The ganglion cell bodies had a large round, eccentric nuclei, and abundant cytoplasm. The thickness of this layer was 9.93 ± 0.85 μm . The micrometrical data observed was lower than the findings of Khaled (2003) in bovine. The non-myelinated axons of the ganglion cells were arranged parallel. They made thick layer, forming the optic nerve fiber layer (Fig. 1). The inner most layer

comprised of the supporting glial (Müller's) cells. The thickness of this layer was $23.32 \pm 0.81\mu\text{m}$. A similar observation was recorded by Khaled (2003) in bovines and Babu *et al.* (2022) in buffaloes.

The inner limiting membrane was laid between the vitreous body and the supporting glial cells of the retina and contained elastic material (Fig. 1) that was in accordance with the reports of Rajathi and Muthukrishnan (2022). The thickness of this layer was $6 \pm 0.3\mu\text{m}$.

CONCLUSION

The basic histological plan of the retina of buffalo was similar to that found in other species. The retina was a delicate innermost membrane extending from the optic nerve's entrance to the pupil's margin. Its inner surface was in contact with the hyaloid membrane of the vitreous humor and its external surface was in contact with the choroid coat. However, the micrometrical observations of all the ten layers of the retina revealed that the thickness of the various retinal layers in buffalo was less than that of bovines and also varied among breed of the same species.

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