Haryana Vet. (October, 2022) **61(SI-2),** 36-39

Research Article

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

JYOTIRMAY ADHIKARY, PARTHA DAS and RAJESH RANJAN[1]*
Department of Veterinary Anatomy, F.V.Sc., WBUAFSc, Kolkata-700037, India
[1]Department of Veterinary Anatomy, College of Veterinary Science, Rewa,
NDVSU, Jabalpur-486001, Madhya Pradesh, India

Received: 13.09.2021; Accepted: 19.10.2021

ABSTRACT

The fibrous tunic of eyeball plays an important role in protecting the eye from external and internal forces, maintaining the shape of the globe as well as helps the retina in proper image formation. Thus, the present study was conducted to elucidate the gross and histological details of the fibrous tunic of eyeball of Murrah buffalo as there is paucity of literature in buffaloes. The results revealed that the anterior 1/5th of the fibrous tunic was composed of cornea and the sclera formed the posterior 4/5th of the fibrous tunic. Cornea was transparent while the sclera was whitish gray in colour. The sclera was thickest at the posterior pole and thin at the sclero-corneal junction. At the sclero-corneal junction it slightly overlapped the cornea.Microscopically, the cornea was avascular and composed of five layers and the sclera was subdivided into three layers. The corneal epithelium was composed of 12-16 rows of epithelial cells layer. The substantia propria or stroma accounted for more than 80% of the thickness of the cornea.The tough layer of the dense irregular connective tissue of the sclera protects the eye and maintained its shape.

Keywords: Buffalo, Cornea, Gross, Histology, Sclera

How to cite: Adhikary, J., Das, P. and Ranjan, R. (2022). Gross and histo-architectural studies on the fibrous tunic of eyeball of Murrah buffalo. *Haryana Vet.* **61(SI-2)**: 36-39.

The vision is the most important sense. For many animals light perception is both the trigger and the controlling sense for much behavior. "Seeing and doing" is much of what an animal does is related to what it sees. Many biological process display circadian rhythmsin activity, which presumably operate to coordinate cellular function with daily environmental oscillations. Many ocular tissues themselves exhibit circadian rhythms to optimize specific processes which require coordination with the light-dark cycle. The globe of eye consists essentially of three coats enclosing the transparent refractive media. The outer-most, protective tunic is made up of the sclera and cornea.

Sclera is opaque and mostly white, while on the other side, the cornea is transparent, although being structurally homologous to sclera. Sclera maintains the shape of the globe, offering resistance to internal and external forces, and provides an attachment for the extraoccular muscular insertions, while the cornea is the second most important structure only after retina in visual function, for without this effect of the cornea no proper retinal image could be formed. In order for light to reach the retina, it is vital that the cornea remain transparent.

Looking to the importance of this fibrous tunic and paucity of literature in buffaloes, this work was designed to explore the gross anatomical and histological aspects of the fibrous tunic of eyeball of buffalo.

MATERIALS AND METHODS

Total pairs of eyeballs from physically healthy adult Murrah buffaloes (*Bubalus bubalis*) of both sexes were collected from the Tangra slaughterhouse, Kolkata, a government authorized abattoir of West Bengal. The samples were preserved in 10% NBF and Hartmann's fixative. After measuring the gross parameters like length, breadth, thickness and colour of the fibrous tunic, the samples were subjected to standard procedure of tissue processing and sectioning. The slides were stained with H & E for routine histomorphology, PAS-AB at pH 2.5 for neutral and acid mucopolysaccharides and Masson's trichrome for collagen fibers (Luna, 1968). H & E stained slides were used for micrometry by Leica Qwin Images Analyzer software in Leica DM 2000 Microscope.

RESULTS AND DISCUSSION

Gross observations

Cornea: The anterior 1/5th of the fibrous tunic was composed of cornea. It was an elliptical, transparent and non-vascular membranous structure (Fig. 1). Anteriorly it was oval in outline and posteriorly it was circular in shape. It was because of that the anterior surface was convex while the posterior surface was concave. The mean thickness of the anterior pole (vertex) was 1.07±0.23 mm and that of the lateral border was 1.11±0.72 mm. Similar observation was cited by Samuelson (1999) in domestic animals but the value was less than the present observation. In contrary to the present findings, Malsawmkima *et al.* (2014) reported a higher thickness of cornea in the centre than at the periphery in Surti buffaloes. The cornea was filled into the groove formed by the sclera at the sclero-corneal junction.

*Corresponding author: rajesh.ranjan837@gmail.com

In the present study, the horizontal and vertical average diametersof the cornea was observed as 28.98± 0.36 mm and 31.56±0.79 mm, respectively indicating that the vertical diameter was greater than the horizontal diameter. Malsawmkima *et. al.* (2014) also reported that the horizontal and vertical diameters were 2.37±0.03 cm and 2.89±0.04 cm in case of Surti buffalo. The trend of observation was similar indicating that the vertical diameter was more than the horizontal diameter but a different calculated value might be due the breed variation. However, in contrary to the present findings, Samuelson (1999) reported that the horizontal diameter was more than the vertical and in most ungulates. The differences between these diameters were much more pronounced, allowing a remarkable horizontal field of view, which was similar with our present observation. It was further supported by the lateral positioning of the eye orbits within their skulls, which was in accordance with our present observation. It was revealed that the cornea was very large, as reported by Pietro *et. al.* (2012) in buffalo. The thickness and the radius of curvature of cornea were also comparatively large than that of other domesticated animals.

Sclera: Sclera formed the posterior 4/5th of the fibrous tunic. The colour of the sclera was whitish gray (Fig. 2). This was in agreement with the observations of Kolb (2007) in bovines.

The sclera was thickest at the posterior pole with mean thickness of 1.14±0.53 mm while at the sclero-corneal junction, it was 0.52±0.03 mm. The values obtained was much lower than the observations of Samuelson (1999) in bovine. Malswamkima *et. al.* (2014) reported that in case of Surti buffalo the peripheral and central thickness of sclera was 445.96±23.05 µm and 856.95±28.68 µm, respectively. At the sclero-corneal junction it slightly overlapped the cornea and there was a shallow groove found externally the "Sulcus sclera" in which the cornea was fitted. Similar observation was noted by Kolb (2007) in bovines.

Its external surface revealed the point of insertion of the ocular muscles and its anterior part was covered by "Conjunctiva sclerae". The tendon of the eye muscle was connected with the dense layer of the connective tissue of sclera. The eye ball and capsule of the tendon rotated together in all direction on a bed of orbital fat. The outer surface of the sclera was covered by membranous inner muscle termed as "Fascia bulbae". This was in accordance with the findings Dyce *et. al.* (1987) in domestic animals.

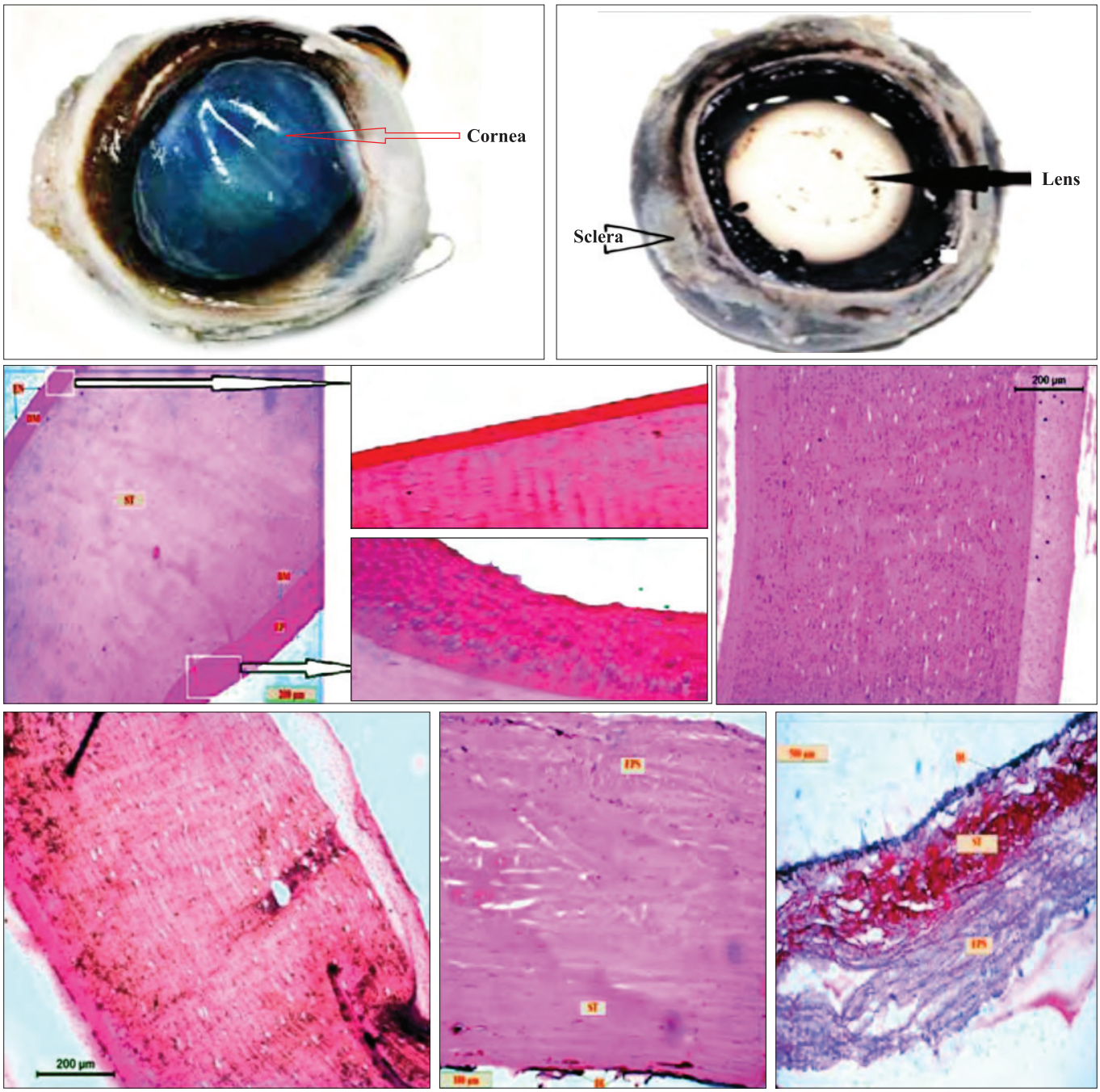
Microscopic observations

Cornea: The buffalo cornea was an avascular structure and composed of five layers: anterior epithelium, sub epithelial basement membrane, substantia propria or stroma, posterior limiting membrane or Descemet's membrane and posterior epithelium or corneal endothelium. Avascularcorneal epithelium was composed of stratified squamous non-keratinized epithelium (Fig. 3).

There were 12-16 rows of epithelial cells observed in the present study (Fig. 3; inset) whereas, Diesem (1975) reported 14 to 18 rows in case of bovine. Malsawmkima *et. al.* (2014) reported that the number of epithelial cell layers were 7 to 12 in Surti buffalo which was not in accordance with our present observation. These cells were situated more in the periphery than center that was similar to the findings of Martin and Anderson (1981) in bovines. The mean thickness of the corneal epithelium was 142.12±1.83 µm. This was not in agreement with the findings of Khaled (2003) in bovine and Malsawmkima *et. al.* (2014) in Surti buffalo.

Bowman's membrane was observed as thin membranous structure separating the epithelium and the substantia propria. It appeared homogeneous, neither fibers nor cell nuclei were distinguished. It was merely a condensation of the next layer. It was more intimately attached to the substantia propria and was considered to be a part of that portion of the cornea. Average thickness of the Bowman's membrane was 12.07±0.84 µm. This was in accordance with the findings of Raghavan and Kacharoo (1964) in ox and Diesem (1975) in bovine. Gelatt (2007) reported absence of this layer in carnivores. Ramakrishna *et. al.* (1997) reported that this layer was not evident in Indian water buffalo.

The substantia propria was composed mainly of collagenous fibers that were arranged in regular layers (Fig. 4). The thickness of the substantia propria was 917.6± 14.6 µm. In contrary to this, Malsawmkima *et al.* (2014) reporteda lower mean thickness of 626.74±12.02 µ in case of Surti buffalo. The substantia propria accounted for more than 80% of the thickness of the cornea. All fibers within a given layer lied flat and parallel to each other, but the orientation of fibers of adjacent layers was oblique to each other. There was some inter-meshing of fibers between adjacent layers, which served to tie the layer together within the stroma. The predominant cell type was the fibroblast which was located within the collagen layers. The fibers were enmeshed in the amorphous ground substance. This ground substance plays essential roles in transparency of cornea. The stroma maintained specific level of hydration to remain transparent. Similar observations were recorded by Raghavan and Kacharoo (1964) in ox, Diesem (1975) in bovine and Dellmann and Collier (1987) in domestic animals. The Descemet's



Figs. 1 to 7. **(1)** Photograph of Eye showing Cornea (arrow). **(2)** Photograph of Eye showing Sclera and Lens; **(3)** Photomicrograph of Cornea showing Corneal Epithelium (EP), Bowman's membrane (BM), Stroma (ST), Descemet's membrane (DM) and Corneal Endothelium (EN) (H&E 10X). **(4)** Photomicrograph of Cornea showing Collagen fiber distribution. (Masson's trichrome 10X); **(5)** Photomicrograph of Cornea showing PAS positivity reactions in various layers. (PAS 10X); **(6 & 7)** Photomicrograph of Sclera of showing Epi Sclera (EPS), Stroma (ST) and Dark Layer (DL) in H&E (20X) and MT (4X) staining, respectively.

membrane was fairly thick, glassy, homogenous layer (Fig. 4). It glistens and broke off at the margin into three sets of fibers: anterior, middle and posterior one. These findings was in agreement with Diesem (1975) in bovine and Slatter (2001) in domestic animals. The thickness of the descemet's membrane was 58.25±0.42 µm. It was similar with the findings of Malsawmkima *et al.* (2014) in Surti buffalo.

The inner most or posterior layer of the cornea was an endothelium layer (Fig. 3). It consisted of a single layer of low cuboidal cells or flattened cells with their nuclei lying parallel to the internal limiting membrane. It was in direct contact with the aqueous humor of the anterior chamber, separated from the substantia propria by the narrow refractile membrane (Descemet's membrane). It was highly refractile, thick amorphous layer. It gave a

PAS-positive reaction (Fig. 5). Average thickness of the corneal endothelium was recorded 3.3±0.32 µm.This was in agreement with the findings Diesem (1975) in bovines, Slatter (2001) in domestic animals and Malsawmkima *et. al.* (2014) in Surti buffalo.

Sclera: Sclera was composed of dense irregular connective tissue (Fig. 7). The collagen fibers were arranged in bundles and were oriented in circular direction around the optical axis. Within the collagen bundle, elastic fibers, elongated fibroblast and some pigmented cells were observed.

The mean thickness of sclera was 942.36±28.88 µm at the center. Malsawmkima *et. al.* (2014) reported that the mean scleral thickness in case of Surti buffalo was 445.96 ±23.05 µ and 856.95±28.68 µ in the periphery and center, respectively, which was found to be less than the present observations. However, Khaled (2003) in bovine eye ball reported that the thickness the sclera was 1132±24.4 µm which was found to be much higher than the present observation.

The sclera was subdivided into three layers. Similar observations were also reported by Dellmann and Brown (1976) in domestic animal, Ramakrishna *et. al.* (1997) in Indian water buffalo and Malsawmkima *et. al.* (2014) in Surti buffalo.

The outermost layer, the epi-scleral tissue (Fig. 6 & 7) consisted of loose fibro-elastic tissue that was continuous externally with dense connective tissue of the tendons of eye muscles. The thickness of this layer was 219.54±6.04 µm. Its deeper surface blended with the middle layer, the sclera proper or stroma, where bundles of collagenous fibers were oriented mainly parallel to the surface with some branching and inter weaving. The thickness of stroma was of this layer was 716.91±3.88 µm.

The innermost layer, termed the lamina fusca or dark layer, was composed of much smaller bundles of collagenous fibers (Fig. 6 & 7). Its mean thickness was 5.91±0.49 µm. The present findings were in agreement with that of Dyce *et. al.* (1987) and Slatter (2001) in domestic animals.

The tough layer of the dense irregular connective tissue of the sclera protects the eye and maintained its shape.

CONCLUSION

The present study revealed that the fibrous tunic of eyeball of Murrah buffalo showed a normal gross and histomorphological disposition as in other domestic animals. Cornea, being transparent and sclera, being opaque have their unique functions required for retinal image formation and maintenance of the shape of globe, respectively. The morphometrical and micrometrical observations varied with the reports in Surti buffalo that might be due to the breed variation. The present gross and histological observations on the fibrous tunic in Murrah buffalo might act as a basic data of eyeball in this breed of buffalo for future research.

REFERANCES

Dellmann, H.D. and Collier, L.L. (1987). Eye. In: Textbook of Veterinary Histology. (3rd Edn.), Editors: Dellmann, H.D., E.M. Brown. Lea & Febiger, Philadelphia, pp. 416-433.

Diesem, C. (1975). Sense Organ and Common Integuments. In: The Anatomy of the Domestic Animals. Revised by R. Getty. W.G. Saunders Company. Philadelphia, London, Toronto, Mexico City, Rode Janise, Sydney, Tokyo.

Dyce, K.M., Sack, W.O. and Wensing, C.J.G. (1987). The Sense Organs. In: Textbook of Veterinary Anatomy. (1st Edn.), W.B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, pp. 328-352.

Gelatt, K.N. (2007). Veterinary Ophthalmology. Australia, Blackwell Publishing Asia, Victoria.

Khaled, A. (2003). Glycohistochemical, immunohistochemical and electron microscopic examination of the bovine eyeball. Thesis submitted to LMU, Munich, Faculty of Veterinary Medicine. pp. 8-119.

Kolb, H. (2007). Gross Anatomy of eye. The Organization of the Retina and Visual System. **24**: 3.

Malsawmkima., Barhaiya, R.K., Vyas, Y.L. and Bhayani, D.M. (2014). Histomorphological study on cornea and sclera of the adult Surti buffalo (*Bubalus bubalis*). *Glob. J. Res. Anal.* **3(11)**: 13-15.

Martin, C.L. and Anderson, B.G. (1981). Veterinary Opthalmology. Gelatt, K.N. (Edt.), (1st Edn.), Lea and Febiger, Bailliere Tindall Publication, London.

Pietro, D., Marco, L., Sebastiano, S. and Giuseppe, L. (2012). Variations in image optical quality of the eye and the sampling limit of resolution of the cone mosaic with axial length in young adults. *J. Cata. Refra. Surg.* **38**: 1147-1155.

Raghavan, D. and Kacharoo, P. (1964). Anatomy of the Ox. With Comparative Notes on the Horse, Dog and Fowl. Indian Council of Agricultural Research. New Delhi.

Ramakrishna, V., Tiwari, G.P. and Ashok, P. (1997). Histomorphology of tunica bulbus oculi in Indian water buffalo-conjunctiva and fibrous tunic. *Kar. J. Agri. Sci.* **10(2)**: 513-516.

Samuelson, D.A. (1999). Ophthalmic Anatomy. Veterinary Ophthalmology (3rd Edn.), Editor: Gelatt, K.N. Lippincott, Williams and Wilknis. A Wolters kluwer Company. Philadelphia. Paltimore. New York. London. Buenos Aires. Hong Kong. Sydney. Tokyo.

Slatter, D. (2001). Structure and function of the eye. In: Fundamentals of veterinary ophthalmology. (3rd Edn.), W.B. Saunders Company, Philadelphia, London, New York, St. Louis, Sydney, Toronto.