“GROSS AND HISTOMORPHOLOGICAL STUDY ON EYE BALL OF THE ADULT SURTI BUFFALO (Bubalus bubalis)”

A
THESIS
SUBMITTED TO ANAND AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF
MASTER OF VETERINARY SCIENCE
IN
VETERINARY ANATOMY AND HISTOLOGY

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ANAND – 388 001 (GUJARAT)
(2014)
(Registration No. 04-1977-2012)
DEDICATED
TO
MY BELOVED
PARENTS
ABSTRACT
ABSTRACT

“GROSS AND HISTOMORPHOLOGICAL STUDY ON EYE BALL OF THE ADULT SURTI BUFFALO (Bubalus bubalis)”

NAME OF STUDENT        MAJOR ADVISOR
MALSAWMKIMA             DR. Y.L. VYAS

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The present study entitled “Gross and Histomorphological Study on Eye Ball of the adult Surti Buffalo (Bubalus bubalis)” was carried out at the Department of Veterinary Anatomy and Histology in collaboration with Department of Veterinary Surgery & Radiology and Department of Veterinary Pathology, College of Veterinary Science & A.H., Anand Agricultural University, Anand, Gujarat. For this study, 20 eye specimens (10 right and 10 left) of buffaloes were used for the sonoanatomy, gross and histomorphological evaluations. The echobiometrical, biometrical and micrometrical measurements of the different parameters of the eye ball including cornea, sclera, choroid, retina and lens were recorded with the help of the scientific weighing balance, Vernier callipers, graduated eye piece and ultrasound machine. The histological sections were stained with Haematoxylin and Eosin Stain for routine staining and Masson’s trichrome stain and Periodic Acid Schiff’s stain for special staining.
The ultrasonography of eye balls showed that the eye balls were appeared as ovoid structures with anechoic contents such as aqueous humour, vitreous body and lens. The cornea, anterior and posterior lens capsule, iris, ciliary body and corpora nigra were appeared as echogenic structures. The overall echobiometrical mean values of the anterior chamber depth, the antero-posterior depth of the lens, the vitreous chamber depth and the antero-posterior depth of both sides of the eye balls were $0.325\pm0.005$ cm, $1.045\pm0.005$ cm, $1.635\pm0.005$ cm and $3.135\pm0.005$ cm respectively.

The overall biometrical mean values of the weight, the antero-posterior axis, the horizontal axis and the vertical axis of both sides of the eye balls were $31.16\pm0.01$ gm, $3.67\pm0.00$ cm, $4.04\pm0.00$ cm and $4.01\pm0.01$ cm respectively.

The sclera was a white tough membrane of the eye ball perforated by the optic nerve at the posterior part. Histologically, it can be subdivided into three layers such as (i) the episclera (ii) the sclera proper and (iii) lamina fusca. The overall micrometrical mean values of the thickness of the sclera at the periphery was $445.96\pm23.05$ $\mu$m and at the center was $856.95\pm33.84$ $\mu$m.

Histologically, the choroid was found to be composed of four layers such as (i) Suprachoroid (ii) Large vessel layer (iii) Tapetum and (iv) Choriocapillary layer. The mean thickness of choroid at the center of the tunic was $76.55\pm3.72$ $\mu$m whereas, it was $48.86\pm1.78$ $\mu$m at the peripheral section of the tunic. The ciliary body was composed of ciliary muscles, collagen fibers, blood vessels, melanocytes, fibroblasts and processes.

The cornea was an elliptical transparent membrane and the anterior surface was convex while the posterior surface was concave. The overall biometrical mean values of the vertical diameter, the horizontal diameter and thickness of both sides of
the cornea were 2.37±0.00 cm, 2.905±0.015 cm and 1.20±0.09 mm respectively. The histological structure of cornea was composed of four corneal layers. These were (i) Anterior epithelial layer (ii) Corneal stroma (iii) Descemet’s membrane (iv) Endothelial layer. The overall micrometrical mean values of the thickness of the epithelial layer, number of the epithelial layers, thickness of stroma, thickness of Descemet’s membrane, thickness of endothelium and total thickness of both the center and periphery of the cornea were 99.29±0.625 µm, 9.35±0.06, 662.45±4.86 µm, 18.705±0.08 µm, 5.015±1.83 µm and 794.05±4.92 µm respectively.

The histological structure of retina was composed of ten layers such as (i) Pigmented epithelium (ii) Layer of rods and cones (iii) External limiting membrane (iv) Outer nuclear layer (v) Outer plexiform layer (vi) Inner nuclear layer (vii) Inner plexiform layer (viii) Ganglion cell layer (ix) Nerve fibers layer (x) Internal limiting membrane.

The thickness of the nerve fibers was greatly increased towards the optic disc. The nerve fibers were converged at the optic disc and passed through a sieve like structure of sclera, known as lamina cribrosa and then formed the optic nerve. The overall micrometrical mean values of the thickness of retina in the center of the tunic was 177.56±10.72 µm and that of retina in the peripheral section of the tunic was 120.24±15.40 µm.

The lens was a transparent, soft and biconvex substance with the convexity more in the posterior surface than that of the anterior surface. The overall biometrical mean values of the weight, thickness and diameter of both sides of the lens were 2.525±0.005 gm, 1.32±0.00 cm and 1.845±0.005 cm respectively. Histologically, it was composed of three components namely (i) capsule, the outermost covering of the
lens (ii) simple cuboidal epithelium and (iii) fibers, which formed the major portion of
the lens.

Almost all the biometrical observations of the eyeball including cornea and
lens were non significantly lower in the right eye balls than that of the left eye balls
and all the micrometrical observations of cornea were non significantly lower in the
center than that of the periphery of cornea. However, the micrometrical observations
of sclera, choroid and retina were non significantly lower in the periphery than that of
the center of the tunics.
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Professor & Head,
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**CERTIFICATE**

This is to certify that the thesis entitled “Gross and Histomorphological Study on Eye ball of the adult Surti Buffalo (*Bubalus bubalis*)” submitted by Malsawmkima (Registration No. 04-1977-2012) in partial fulfillment of the requirements for the degree of Master of Veterinary Science (Veterinary Anatomy and Histology) of Anand Agricultural University is a record of a bonafide research work carried out by him, under my supervision and guidance and the thesis has not previously formed the basis for award of any degree, diploma or the similar title.

Malsawmkima has completed his course work and has passed the preliminary examination.

Place : Anand
Date : / /2014

(Y.L. VYAS)
Major Advisor
CERTIFICATE

This is to certify that I have no objection for providing to any scientist only one copy of any part of this thesis at a time through reprographic process, if necessary for rendering reference service in a library or documentation center.

(MALSAWMKIMA)

Research scholar

Station: Anand

Date: / /2014

(Y.L. VYAS)

Major Advisor
Acknowledgement

Finally, I came to the end part of my journey of M.V.Sc. Degree by the Grace of God, His uncountable blessings which I received through my teachers, my friends and my family that had been leading me to be in this position. So, I would like to give my sincere thanks to all of them through this humble acknowledgement.

First of all, I give my heartiest thanks to Almighty God, as without his mercy and help, accomplishment of my work & preparation of this manuscript would not have been possible.

At this moment, indeed, I have great pleasure in expressing my deep sense of gratitude and appreciation to my Major Advisor, Dr. Y.L. Vyas, Professor and Head, Department of Veterinary Anatomy and Histology, for his judicious and constantly inspiring guidance with constructive criticism, persistent encouragement, active persuasion, diligent efforts and caring attitude throughout the course of my study and research work.

I express my heart-felt gratitude to my Minor Advisor, Dr. K.S. Prajapati, Professor and Head, Department of Veterinary Pathology, for providing me spirited guidance and critical counsel as the occasion required, I also thanks him for his constructive suggestions throughout the course of my study.

I am equally grateful to the members of my advisory Committee, Dr. D.B. Patil, Professor and Head, Department of Veterinary Surgery and Radiology and Dr. K.M. Panchal, Professor, Department of Veterinary Anatomy and Histology for their wise suggestions, constant support and cooperation during the course of this research.

I also express my sincere thanks to Dr. A.M. Thaker, Dean, College of Veterinary Science and Animal Husbandry, Anand, for providing me all the
necessary facilities during the research work and an opportunity to pursue my higher studies from such an esteemed institute of Gujarat state.

I am really very thankful to Dr. D.M. Bhayani, Professor, Department of Veterinary Anatomy and Histology, for his excellent cooperation, help and technical advice during my research work.

I extend my heartfelt gratitude to Dr. S.C. Dubal, Professor, Department of Veterinary Anatomy and Histology, Dr. (Mrs) Sweta P. Pandya, Associate Professor, Department of Veterinary Anatomy and Histology, Dr. D. J. Ghodasara, Associate Professor, Department of Veterinary Pathology, Dr. P.V. Parikh, Professor, Department of Veterinary Surgery and Radiology, Veterinary College, Anand for their valuable suggestions, support and excellent cooperation during the entire work.

I would like to extend my heartfelt gratitude to Dr. Varsha, Dr. Rajwanti, Dr. Divyesh and Dr. Urja for their valuable help in my research work.

I am very thankful to Mrs. Himani Patel, Lab Technician, Department of Veterinary Pathology, for her cooperation in preparing some of the histological sections. I am also thankful to Mr. Amitbhai Patel, Clerk, P.G.T, Dept. of Veterinary Pathology, for his cooperation and kind suggestions.

I acknowledge my thanks to the laboratory technician and supporting staff of Dept. of Veterinary Anatomy and Histology, Mehtabhai, Prakashbhai, Shanabhai, Babubhai, Ambalal, Rambhai (retired lab. technician) for their co-operation throughout the course of study.

I am happy to acknowledge the support of my batchmate, Dr. Rakesh Barhaiya, for his valuable help and cooperation starting from the beginning to till completion of my research work.

I am also happy to acknowledge the support of my batchmates Dr(s) Ishita, Sharadindu, Bhumit, Jainudin, Sunanda, Priya, Satish, Nakrani, Dinesh, Ankit, Sunil, Yamini, Shivani, Paresh, Jeetu, Suchit, Vandeep,
**Yogesh and Ravi** for their cooperation and help rendered during the difficult times.

I give my heartfelt gratitude to my respected seniors **Dr(s). Theodore, Waseem, Menguzeno, Arpan, Subhash, Shyam, Bhadesya, Chotur and Khanvilkar** for their kind help and support during the entire period of my study in this place.

I feel pleasure in expressing my thanks to my juniors **Dr(s). Aarti, Vinay, Abhilash, Tejas, Sumana and Mr Subham** for their affection and helps during my study and research period.

I give my heartfelt thanks to my parents, brothers and sisters for all your encouraging attitude and ever available help that stirred me to put in my best. Thank you for all your supports which helped me to reach this position.

This thesis is a part of my individual success, but is more a part of the success of the mentioned individuals and others. Their ability to persuade my courage and encourage my determination have developed me to the person I am today, and I thank them for this.

My eternal thanks to all of you!

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**Place : Anand**

**Date : / /2014**
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INTRODUCTION
CHAPTER - 1
INTRODUCTION

“The Lamp of the body is the Eye”
*Matthew 6 : 22*

The water buffalo or domestic Asian water buffalo (*Bubalus bubalis*) is of two types, termed Swamp and Riverine types (Cockrill, 1977). Riverine buffaloes are distributed in India, Pakistan, Sri Lanka and some European countries such as Italy, Bulgaria, Greece and Yugoslavia. Swamp buffaloes are spread in Far East Asia including China, Indo-China, Indonesia, Philippines and Thailand (Ross, 1975). The Surti is a breed of water buffalo found mainly in Gujarat, India and is of medium size and docile temperament (Anonymous, 2013).

India is the world’s largest single milk producer in the world and buffalo milk is contributing more than 50 % of the overall milk production, making it one of the world’s top producers of this alone (FAOSTAT, 2012). The buffalo meat is also contributing 24.54 % of the total meat produced in the country (FAO, 2008). So, the importance of buffalo in dairy and meat industry is very high but, still research related to buffalo is very insufficient especially in the sense organs like eyes.

The eye is an important sensory organ designed for vision. By means of the refracting system, an inverted image of the object is formed in the retina. It may be considered as an optical instrument acting like a camera, relaying information received in the form of light to the brain. Since the well being and performance of the animals is directly related to proper vision, the animal with poor vision usually results in poor performance and also prone to accidents and dangerous violent activities.
The eye or organ of vision in the broader sense of the term comprises the eyeball or globe of the eye, the optic nerve, and certain accessory organs associated therewith. The accessory organs are the orbital fasciae and muscles, the eyelids and conjunctiva, and the lacrimal apparatus (Sisson and Grossman, 1950).

Basically, the eye is composed of a lens and a wall that is divided into three layers: an outer fibrous tunic, a middle vascular tunic, and an inner nervous tunic. The fibrous tunic is divided into the posterior, opaque sclera and the anterior, transparent cornea. The vascular tunic includes the choroid, ciliary body and iris. The nervous tunic consists of a ten-layered, photosensitive retina and a bilayered, non-photosensitive portion (Bacha and Bacha, 2000).

The sclera is a white, tough layer of dense irregular connective tissues that protects the eye and maintains its form (shape). The anterior portion of the fibrous tunic (i.e. cornea) is transparent, thus enabling light to pass through, and is shaped in a manner that makes it a powerful lens that refract light rays centrally, toward the visual axis of the eye. The cornea is one of the most densely innervated tissues in the body and is richly supplied by sensory and autonomic nerves fibers (Muller et al., 2003).

The middle layer is tunica vasculosa (uvea) which is heavily pigmented and vascularized. It includes the choroid, ciliary body, and iris (Bacha and Bacha, 2000).

Choroid is pigmented, highly vascular layer providing nutrition to other structures. Its deepest layer is the tapetum, which is a reflective surface designed to pop up the light entering in the retina, thus increasing the dim light vision (Leite et al., 2013). The ciliary body is a circumferential thickening of the vascular tunic that gives rise to many fine suspensory ligaments that support the lens. It produces aqueous humour and vitreous humour, and is involved in lens accommodation, as its muscle
fibers stretch the lens into a flatter shape, allowing distant vision. The iris is the most anterior portion of the vascular tunic and is pigmented and contractile for pupil size.

The third innermost layer is retina. The retina is a multilayered extension of the brain that play key role in vision (Komaromy, 2010). Briefly, the retina consists of various cell types arranged in eight layers and two membranes (Shara et al., 2013). Visual perception is a sensory process initiated at the retina, and completed in the cerebral cortex. Two main functions are currently performed by the retina: 1) the initial conversion of light energy into electric signals, photo-transduction, which is carried out by photoreceptors; 2) a series of physiological processes performed by retinal interneurons (bipolar, horizontal and amacrine cells), in order to encode the different attributes of the visual stimuli (shape, movement and color) in electrical signals (Germain et al., 2010). Any changes in its structure may lead to temporary or permanent blindness.

The lens is transparent, biconvex, fine tuning refractive structure that focuses sharp image on the retina for acute vision. It is situated between the iris and the vitreous body. It is suspended by the zonular fibers arising from the ciliary body and attaching to the lens capsule at the lens equator. It consists of the lens capsule, lens epithelium, and lens fibers. The lens is completely enclosed within a thick, PAS-positive elastic capsule. It has elastic property but no elastic fibers (Hogan et al., 1971). The lens capsule itself consists of fibrils and basement membrane that are laminarly arranged. Inside the anterior capsule is a single layer of lens epithelium which is cuboidal to squamous centrally, become columnar near the equator. The lens epithelium lines only the anterior surface the lens capsule (Gelatt, 2007).

The cornea and the lens act as refractive media because of their transparency and curvature. Cornea, being central and outermost part of the eye ball, is frequently
subjected to get injury causing ulcer, keratitis, opacity etc. resulting pain and discomfort to the animal.

The eye contains three fluid-filled regions. Firstly, the anterior chamber which is bordered by the cornea, iris and lens. Secondly, the posterior chamber which is located between the iris, lens, zonular fibers and ciliary processes. Both of these chambers contain aqueous humor. Thirdly, the most posterior compartment or the cavity of the vitreous humor which is lying behind the lens (Bacha and Bacha, 2000).

With the introduction of ultrasonography, it becomes possible to diagnose certain disease conditions of the eyes without causing pain to the animals. Ocular ultrasonography (USG) is safe noninvasive technique to evaluate the intraocular and retrobulbar tissue of opaque eyes (Nyland and Mattoon, 1995). Two dimensional USG allows evaluation of structure such as cornea, the anterior chamber, the iris, the ciliary body, the lens, the vitreous chamber and the posterior section of the bulbar wall (Nautrup and Tobias, 2007). Ocular biometry is one of the methods to measure the axial dimensions of the eye and determine the position of intraocular components by USG. Ocular biometry has been useful for the assessment of certain pathologic abnormalities such as phthisis bulbi, microphthalmia, scleral ectasia and congenital glaucoma (Brando, 2007; Potter, 2009). Knowledge of the dimensions of the optical components is required for better understanding of clinical problems in vision.

A sound knowledge of normal gross anatomical and histological features including possible individual variations will greatly assist in recognizing pathology thus providing more accurate diagnosis and will aid in accurate tissue sampling. A number of studies have been carried out on the other system of buffalo but study on the eye ball is very little. There is hardly any literature on the anatomy of the buffalo in general and on the anatomy of the eye ball in particular. And also the study /
research related to gross and histomorphology on the eye ball of buffalo is very few in India and also the breed wise study is not found in the available literature reviewed. Hence, with these views in mind, the research work is carried out in the Surti buffalo with the following objectives and it is hoped that these studies would also go along way in helping the surgeons in refining ophthalmic surgery in the buffalo:

1. To evaluate sonoanatomy and ocular echobiometrical dimensions of the eye ball by using two-dimensional ultrasonography.
2. To study gross morphology of the eye ball.
3. To study gross morphology of the cornea.
4. To study histomorphology and micrometry of the cornea.
5. To study histomorphology and micrometry of the sclera, choroid and retina.
6. To study histomorphology of ciliary body and iris.
7. To study gross morphology and histomorphology of the lens.
REVIEW
OF
LITERATURE
2.1 ULTRASONOGRAPHY OF EYE BALL

2.1.1 Sonoanatomy and Ocular ultrasonography

Whitcomb (2002) studied the normal sonoanatomy of the eyes in horses. He reported that the anterior chamber and vitreous chamber of the eye balls were filled with anechoic fluid. The iris and ciliary body were seen as echoic linear structure extended from the peripheral globe towards the lens. The corpora nigra or iridica granules were seen as an echogenic sound of tissue on the anterior surface of the dorsal iris. The normal lens was anechoic and only the anterior and posterior reflection of the lens capsule can be seen in normal eye. The normal retina could not be differentiated from the choroidal layer. The optic nerve demonstrated a cone shape appearance and homogenous echogenicity. The optic nerve was surrounded by the extraocular muscles, which demonstrated a hypoechoic and mottled echogenicity.

Spaulding (2008) reported the ultrasound imaging of eyes in dogs. In normal dog eye, the three cavities (anterior chamber, posterior chamber and vitreous body) have anechoic appearance. The first highly reflective line was the corneal surface. The anterior chamber was delineated by the cornea, the iris and the central lens capsule. Posterior to the lens and extending to the posterior aspect of the globe is the vitreal body, which filled the vitreous cavity. Retinal wall was hyperechoic but could not be differentiated from the other two layers.

Assadnassab and Fartashvand (2013) studied the ultrasonographical appearance of normal eyes of live buffalo. They observed that on B-scan images, the
buffalo eyes appeared as well-defined, ovoid structures. The cornea appeared as a double-peaked echo (2 convex interfaces) with a central, narrow anechoic space. The anterior lens capsule appeared as a convex echogenic line separated from the concave echogenic line of the posterior lens capsule by the anechoic lens. The iris and ciliary body were observed as linearly shaped moderately echoic structures. The iris was identified immediately adjacent to the anterior lens capsule with the thicker, irregular ciliary body lying peripheral to it. The anterior and posterior chambers of the aqueous appeared as a single, anechoic space. The vitreous chamber was imaged as a homogeneous, anechoic region between the posterior lens capsule and ciliary body anteriorly and the posterior ocular wall. The posterior ocular wall had a good echogenicity encountered. In their study, it was not possible to identify individual retinal, choroidal, or scleral layers.

### 2.1.2 Echobiometry

Ribeiro *et al.* (2010) determined the echobiometric findings in the eyes of 30 adult goats. Mean and standard deviation from the ocular structures of the male & female goats were 3.46±0.55 & 3.33±0.46 (anterior chamber depth); 8.60±0.34 & 8.65±0.39 (lens thickness); 11.34±0.61 & 11.39±0.66 (vitreous chamber depth); and 23.43±0.92 & 23.39±0.86 (axial globe length) mm respectively.

Patil *et al.* (2011) reported that the mean anterior chamber depth (ACD), crystalline lens thickness (CLT), vitreous chamber depth (ACD), axial globe length (AGL) of right & left eye in male horses were 3.96±0.13 & 4.22±0.17, 11.05±0.13 & 11.45±0.16, 20.15±0.30 & 19.8±0.29, 35.28±0.34 & 35.50±0.32 mm respectively.

Assadnassab and Fartashvand (2013) reported the different measurements of normal eyes of live buffalo. On comparing these measurements, the antero-posterior depth of the lens, vitreous chamber, and axial length of the globe on the left side were
greater than that on the right. The antero-posterior depth of the lens of left and right were 1.135 ± 0.052 cm and 1.132 ± 0.053 cm respectively, vitreous chamber depth of the left and right were 1.677 ± 0.042 cm and 1.670 ± 0.040 cm respectively, and axial length of the globe on left and right were 3.297 ± 0.037 cm and 3.292 ± 0.037 cm respectively. The anterior chamber depth of the left side was less than that of the right side in each of the buffaloes. The anterior chamber depth of the left and right sides were 0.287 ± 0.015 cm and 0.291 ± 0.014 cm respectively, but these differences were reported not to be statistically significant.

2.2 EYE BALL

2.2.1 Biometry of the eye ball

Smythe (1956) quoted the measurements of various axes of eye ball in ox as follow:

Antero-posterior axis : 3.53 cm
Vertical axis : 4.08 cm
Horizontal axis : 4.19 cm

Prince et al. (1960) mentioned the measurements of various axes of eye ball in ox as:

Antero-posterior axis : 3.40 to 3.70 cm
Vertical axis : 3.70 to 4.20 cm
Transverse axis : 3.80 to 4.30 cm

Martin and Anderson (1981) quoted the following measurements of the different parameters of the eye ball in cattle as:

Antero-posterior axis : 3.53 cm
Vertical axis : 4.08 cm
Horizontal axis : 4.19 cm
Weight of both eyes : 65.00 gm

Panchbhai et al. (1988) reported the mean values of the following measurements of the different parameters of eye ball in buffalo calves as below :

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antero-posterior axis</td>
<td>3.19 cm</td>
</tr>
<tr>
<td>Vertical axis</td>
<td>3.45 cm</td>
</tr>
<tr>
<td>Horizontal axis</td>
<td>3.37 cm</td>
</tr>
<tr>
<td>Weight of eye ball</td>
<td>21.41 gm</td>
</tr>
</tbody>
</table>

Banubakode (1992) reported the mean values of the following measurements of the different parameters of the eye ball in cattle as :

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric axis</td>
<td>3.27 ± 0.02 cm</td>
</tr>
<tr>
<td>Vertical axis</td>
<td>3.23 ± 0.08 cm</td>
</tr>
<tr>
<td>Horizontal axis</td>
<td>3.55 ± 0.041 cm</td>
</tr>
<tr>
<td>Weight of eye ball</td>
<td>24.17 ± 0.48 gm</td>
</tr>
</tbody>
</table>

Gelatt (2007) mentioned the different dimensions of eye ball in different domestic animals. The meridional A-P axis, equatorial axis and horizontal axis of the eye ball were 35.34 mm, 40.82 mm, 41.90 mm respectively, in cow.

Khaled and Abdalla (2013) reported that the average weight of eye ball was 35.30 ± 2.69 gm in buffalo.

2.3 CORNEA

2.3.1 Gross morphology & Biometry

Sisson and Grossman (1950) mentioned that cornea was transparent, colourless and non-vascular. It was oval in outline, the long axis being transverse and the broad end medial. The anterior surface was convex and was more strongly curved than the sclera.
Smythe (1956) quoted the work by Henderson wherein the transverse and the vertical axis of the cornea were recorded as 2.9 cm and 2.4 cm respectively, in cow.

Prince et al. (1960) recorded the ranges of the horizontal and the vertical axis of the cornea as 2.7 cm to 3.2 cm and 2.2 to 2.4 cm respectively, in cattle.

Martin and Anderson (1981) quoted the following measurements of the cornea in cattle as:

- Width of cornea : 3.05 cm
- Height of cornea : 2.35 cm

Panchbhai et al. (1988) measured the height and the width of the cornea in 17 buffalo calves and recorded that the mean height (vertical diameter) and the mean width (horizontal diameter) as 2.05 cm and 2.53 cm respectively.

Banubakode (1992) recorded the mean height (vertical diameter) and the mean width (horizontal diameter) as 2.42 ± 0.02 cm and 3.25 ± 0.03 cm respectively, in cattle.

Gelatt (2007) described that cornea was elliptical in shape, with a horizontal diameter being greater than the vertical which allowing for a remarkable horizontal field of view. The corneal thickness varied from species to species, from breed to breed and from individual to individual. He mentioned that the thickness of cornea at center and at edge as 1.5-2.0 mm and 1.5-1.8 mm respectively, in cow.

Faber et al. (2008) reported that corneal thickness of pig as 0.666 mm centrally, 0.657 mm nasally (medial), 0.713 mm inferiorly, 0.669 mm temporally, and 0.714 mm superiorly (mean values). The corneal diameters were also measured as 14.9 mm horizontally and 12.4 mm vertically (mean values).

Ghosh (2012) mentioned that cornea was a transparent membrane, convex in front and concave behind in cattle. The central part was thinner than the peripheral part.
2.3.2 Histology

Prince et al. (1960) mentioned that the histological structures of the cornea consisted of five layers in cattle. They mentioned that the first layer of cornea was composed of stratified squamous epithelium in which the basal rows of cells were of columnar type. Beneath the epithelium was the Bowman’s membrane. Next to the Bowman’s membrane, there was a layer of substantia propria made up of modified fibrous connective tissue. Next to the substantia propria they observed Descemet’s membrane. The most posterior stratum was of endothelium which consisted of a single row of flattened cells.

Martin and Anderson (1981) observed four corneal layers in some of domestic animals. These were (1) Anterior epithelium (2) Stroma (3) Posterior limiting membrane (Descemet’s membrane) and (4) Posterior epithelium (endothelium). They noted that the additional layer i.e. the anterior limiting membrane (Bowman’s membrane) was present in cattle but was indistinct histologically in other domestic animals.

Ross and Edward (1985) described the histological structure of cornea in human beings. They observed five corneal layers viz. (1) Epithelium (2) Bowman’s Membrane (3) Substantia Propria (4) Descemet’s Membrane and (5) Endothelium. They noted that the epithelium was of stratified squamous non-keratinized type and made up of five cell layers with the basal row of columnar cells. They reported that the under surface of the corneal epithelium was smooth and homogeneous called as Bowman’s membrane. They observed that the substantia propria was composed of regularly arranged sheets or lamellae of collagenous fibers and fibroblast. Further they
noted that the descemet’s membrane was made up of homogeneous materials and the endothelium was composed of a single layer of cuboidal cells.

Banubakode (1992) studied the histological structures of cornea in cattle and observed five layers as above. He reported that the epithelium was stratified squamous non-keratinized type and composed of 7 to 12 rows of epithelial cells. Bowman’s membrane was homogeneous in appearance and seen to be continuous with the substantia propria, which constituted the major part of the cornea. The substantia propria was composed of regularly arranged sheets or lamellae of the collagen fibers. The descemet’s membrane was homogeneous in appearance and most posterior layer, the endothelium was composed of a single row of flattened polygonal cells.

Ramkrishna *et al.* (1997) studied the histomorphological structures of cornea in Indian water buffalo. They reported that the cornea showed increased thickness centroperipherally, thus thickest at the cornea-scleral junction. The cornea consisted of four layers viz. (1) Anterior corneal epithelium (2) Substantia propria (3) Descemet’s membrane and (4) Corneal endothelium. According to them, the anterior corneal epithelium was composed of stratified squamous non-keratinized epithelium having 5-10 layers. They reported that the subepithelial basal lamina was not evident. Substantia propria was more compacted towards the epithelium and consisted predominantly of collagen and elastic fibers with fibroblast cells running parallel to the surface of cornea. Descemet’s membrane was a highly refractile, eosinophilic, homogeneous, uniformly thick membrane. Corneal endothelium consisted of a single layer of low cuboidal cells with round or elongated vesicular nuclei.

Khaled (2003) studied the microscopical anatomy of the bovine eyeball. He reported that the cornea was composed of five layers. He reported that the corneal
epithelium was of stratified squamous epithelium consisting of 14-17 layers of epithelial cells. The next membrane, membrane of Bowman, was prominent in bovine cornea. The substantia propria was composed of collagenous fibers arranged in regular layers parallel to each other. Descemet’s membrane was a fairly thick, glossy, homogeneous membrane and consisted mainly of collagen fibers. The corneal endothelium consisted of a single layer of low cuboidal cells or single layer of flattened cells with the nuclei lying parallel to the Descemet’s membrane.

Gelatt (2007) observed the microscopic structure of the cornea in domestic animals and reported that cornea consisted of four layers, and sometimes five layers. The corneal epithelium covered the anterior corneal surface, being non keratinized stratified squamous. Beneath the anterior epithelium there was a basement membrane. The substantia propria was composed of thin collagen fibrils that was uniformly positioned into lamellae and transverse the full diameter of the cornea. Bowman’s layer was not seen in most animals as described in primates. Descemet’s membrane was a homogenous, acellular membrane forming an inner protective boundary within the cornea. The corneal endothelium was a single layer of flattened cells lining the inner cornea.

2.3.3 Micrometry

Prince et al. (1960) mentioned that the total thickness of the cornea in cattle was within the range of 750 to 850 µ. They recorded the thickness of the following individual layers as follow:

- Epithelial layer: 90 µm
- Descemet’s membrane: 10 to 25 µm
- Endothelial layer: 6 µm
Bloom and Fawcett (1962) noted that the total thickness of the cornea was within the range of 0.8 to 0.9 mm in human beings. They also recorded the thickness of the following layers as follow:

- Epithelial layer: 50 µm
- Descemet’s membrane: 5 to 10 µm

Diesem (1977) observed that the thickness of the cornea in bovine varied from 1500 to 2000 µm. According to him, there were 14 to 18 rows of cells in epithelial layer. He noted that the thickness of the endothelial cell layer of cornea was 6 µm.

Camber et al. (1987) worked on the histology of pig cornea. They noted that the number of cell layers in the epithelium was 17 to 23. They also calculated the mean thickness of the cornea as 722 µm.

Banubakode (1992) reported the mean value of the thickness of the cornea in cattle as 933.72 ± 15.35 µm. He reported that the mean value of the thickness of the different layers of the cornea as follow:

- Epithelial layer: 72.08 ± 2.90 µm
- Number of epithelial cell layers: 9.28 ± 0.17
- Endothelium: 2.22 ± 0.067 µm

Khaled (2003) reported that the mean value of the thickness of the different layers of bovine cornea as follow:

- Corneal epithelium: 98 ± 1.5 µm
- Substantia propria: 580 ± 4.0 µm
- Descemet’s membrane: 30 ± 1.0 µm
- Corneal endothelium: 8 ± 0.3 µm

2.4. SCLERA
Sisson and Grossman (1950) mentioned that sclera was a dense fibrous membrane which formed about four-fifths of the fibrous tunic. It was the thickest in the vicinity of the posterior pole, thin at the equator and increased in thickness towards the junction with the cornea. It was generally white, but may have a bluish tinge in its thinnest parts. Its external surface furnished the insertion to the ocular muscles and was covered by the conjunctiva sclerae in its anterior part. They mentioned that the thickness of sclera was 2.00 mm at the posterior pole and 0.40 mm at the equator in horse.

Dellmann (1993) mentioned that sclera was a white, tough layer of dense irregular connective tissue. Bundles of collagen fibers contained a few elastic fibers and elongated fibroblast as well as melanocytes in some areas, and arranged paralleled to the surface of the globe. These bundles were intricately interwoven and arranged predominantly in an equatorial direction near the junction between sclera and cornea, the so-called limbus. In the layer of the sclera adjacent to choroid, fibroblasts and melanocytes were more numerous and this layer was referred to as the lamina fusca sclerae. The optic nerve exit the eye through numerous perforation in a disk like area referred to as the area cribrosa sclerae.

Ramkrishna et al. (1997) studied the histomorphological structures of fibrous tunic of eye ball in Indian water buffalo. They reported that the sclera was fibrovascular and was divisible into episclera, sclera proper and lamina fusca. The episclera was loose and vascular having fibrous connective tissue. The sclera proper was made of dense regular connective tissue with few elastic fibers and fibroblast cells. The fibrous tissue bundles were arranged at right angles to each other but parallel to the surface of the eye ball. Adjacent to the choroid, the pigment cells formed lamina fusca.
Khaled (2003) reported that in the bovine eyeball, the sclera consisted of flat ribbons of collagenous bundles running in various directions with fine elastic nets, fibroblasts and occasional melanocytes between them. He noted that the sclera can be subdivided into three layers: the outermost layer, the episcleral tissue (52 ± 1.5 μm), consisted of loose fibroelastic tissue. The middle layer, the sclera proper (1036 ± 20.7 μm), bundles of collagenous fibers were oriented mainly parallel to the surface but with some interweaving. The innermost layer i.e. lamina fusca or dark layer (44 ± 2.2 μm), was composed of much smaller bundles of collagenous fibers.

2.5. CHOROID

Sisson and Grossman (1950) mentioned that choroid was a thin membrane lying between the sclera and retina. The general coloured of the choroid was a dark brown, but an extensive semilunar area a little above the level of the optic papilla had a remarkable metallic luster, and was termed the tapetum of choroid.

Prince et al. (1960) mentioned that histologically the choroid in domestic animals was made up of four layers which were suprachoroidea, a layer of large blood vessels, choriocapillaries (layer of small vessels) and Bruch’s membrane. They noted that the suprachoroidea layer was an avascular membrane of elastic fibers, pigmented connective tissue and fine transparent lamellae, with fibroblast on their surface. A layer of large blood vessels was very prominent. Bruch membrane was also noted rather difficult to identify except in the region where tapetum was thickest. The tapetum was a reflecting layer of tissue which was fibrous in ungulates. It was situated behind the retina between the choriocapillary and large blood vessels layer of the choroid.
They mentioned that the thickness of the choroid in cattle was about 100 to 160 µm excluding a tapetum. The thickness of tapetum was from 10 µm at the periphery to 50 µm at the center.

Dellmann (1993) mentioned that choroid was thick, highly vascularized layer that was continuous with the ciliary body stroma anteriorly and extended posteriorly around the globe in domestic animals. The choroid was subdivided into five layers which were suprachoroid layer, vessel layer, tapetum lucidum, choriocapillary layer and basal complex. The suprachoroid layer was a loosely structured consisted of bundles of collagen fibers and some elastic fibers. The tapetum was located mainly in the dorsal half of the fundus of the eye. In herbivores the tapetum was fibrous, consisting of intermingling collagen fibers and few fibroblast. The choriocapillary layer was a dense network of capillaries immediately adjacent to the pigmented epithelial layer of the retina. The basal complex (Bruch’s membrane) separated the choroid from the retina.

Ramkrishna et al. (1997) reported that the choroid of Indian water buffalo was highly vascular and comprised of five layers which were (1) Suprachoroid layer (2) Vessel layer (3) Tapetum fibrosum layer (4) Choriocapillary layer and (5) Basal complex or Bruch’s membrane. They noted that the suprachoroid layer consisted of the collagen and elastic fibers along with pigment cells. The vessel layer was composed of very large blood vessels separated by the suprachoroidal stroma and the large vessels appeared to be veins. The tapetum fibrosum layer was fibro vascular and the thickness was inconstant due to overlying vessel layer and the arterioles surrounded by pigment cells were seen in this layer. The fourth layer i.e. choriocapillary layer, consisted of a single layer of capillaries next to the tapetum and also the capillaries were arranged in a continuous chain form. The basal lamina of
these capillaries appeared to be fused with Bruch’s membrane. The basal complex or Bruch’s membrane was found to be sandwiched between the chorio-capillary layer and pigment epithelium of retina.

Khaled (2003) reported that in the bovine eyeball, the choroid can be subdivided into four layers such as (1) the suprachoroid layer (46 ± 1.5 μm) consisted of bundles of collagen. They were separated by numerous spaces, the perichoroidal spaces, and were continuous with the connective tissue of the sclera. (2) The vessel layer consisted of intercrossing large (72 ± 2.5 μm) and medium sized (40 ± 1.4 μm) arteries and veins, separated by loose connective tissue stroma rich in chromatophores. It contained strands of smooth muscle cells. The tapetum was fibrous, consisting of dense regular connective tissue fibers. The choriocapillary layer (50 ± 1.5 μm) contained a dense network of capillaries. It was immediately adjacent to the pigmented epithelial layer of the retina. The basal complex, also referred to as Bruch’s membrane (0.6 ± .005 μm) separated the choroid from retina.

Gelatt (2007) described the histological structure of choroid in domestic animals. He mentioned that choroid had externally to internally four layers which were suprachoroidea, stroma with large vessels, stroma with medium sized vessels and tapetum, and choriocapillaris. The suprachoroidea was composed of elastic, heavily pigmented connective tissue that formed a transition between the sclera and the choroid. The next layer was a vascular large vessels layers which were embedded in loose connective tissue containing melanocytes and fibrocytes. A small layer of medium sized vessels and pigmented reticular connective tissue lied internally to the large vessels layer. In most domesticated animal, the dorsal portion of the choroid at the medium sized vessel layer contain a layer of reflective tissue called tapetum lucidum. The tapetal layer was composed of regularly arranged collagenous fibers in
cattle. The choriocapillaris was the innermost layer of choroidal vessels, forming a thin layer of capillaries which was separated from the retinal pigmented epithelium (RPE) by a basement membrane complex known as Bruch’s membrane.

Ghosh (2012) mentioned that choroid was a dark coloured membrane. The choroid of animals, specially those of nocturnal habit, present a reflecting structure, the tapetum, which gives a greenish glare at night.

2.6 CILIARY BODY

Ramkrishna et al. (1997b) reported that in Indian water buffalo, the ciliary body presented ciliary muscles and processes. The smooth muscle fibers were parallel to sclera, meridional in arrangement with few circular fibers. The basal plate presented connective tissue core with large blood vessels. The ciliary processes consisted of numerous thin folds covered by two layer stratified cuboidal epithelium, whose superficial cells were non pigmented while the deeper tall columnar were deeply pigmented.

Khaled (2003) reported that in the bovine eyeball, the ciliary body consisted of the following layers: (1) the supraciliaris layer consisting of bundles of collagen and some elastic fibers with fibroblasts, numerous flat melanocytes, some smooth muscle cells and occasional macrophages. (2) The stroma of the ciliary body contained a large number of blood vessels, arteries and veins. (3) The Bruch’s membrane of the ciliary body was continuous with the Bruch’s membrane of the choroidea and extended anteriorly to the root of the iris. (4) The pigmented epithelial layer was the continuation of the pigmented epithelium of the retina. It was composed of simple cuboidal or low columnar cells with rounded nuclei. (5) The nonpigmented epithelial layer was the internal cellular lining of the ciliary body with cuboidal or low columnar cells contain oval nuclei. Each ciliary process consisted of a central core of connective
tissue stroma and blood vessels covered by a double layer of epithelium: an inner, pigmented, cuboidal epithelium and an outer, non-pigmented cuboidal epithelium.

2.7 **IRIS**

Sisson and Grossman (1950) mentioned that iris was a muscular diaphragm placed in front of the lens, and was visible through the cornea. It was pierced centrally by an elliptical opening called the pupil. The upper part of pupillary border bear in its middle several black masses of variable size, termed the granula iridis or corpora nigra; similar but much smaller projections may be seen on the lower margin of the pupil.

Ramkrishna *et al.* (1997b) reported that in Indian water buffalo, the thickness of the iris was decreased towards the free margin. The stroma was composed of loose connective tissue with blood vessels, melanocytes and fibroblasts. The anterior surface consisted of pigmented cells and the posterior surface showed two layered pigmented epithelium. The superficial non pigmented epithelium of the ciliary processes formed superficial layer of cells, however which were heavily pigmented while the deeper cells were less pigmented. No iridial granules (granula iridica) were reported to be seen along the pupillary margin of the iris. The sphincter muscles were seen along the pupillary margin, while the dilator muscles were seen at the base of the ciliary processes.

Khaled (2003) reported that histologically the bovine iris consisted of three layers such as (1) An anterior epithelial layer which consisted of fibroblasts and melanocytes and was continued across the iridocorneal angle into the posterior epithelium of the cornea. (2) A middle layer of connective tissue stroma, which contained two smooth muscles (dilatator and sphincter pupillae muscles) and (3) The posterior layer of the pigmented epithelium. He also reported the presence of granula
iridis (iris granules) at the pupillary edge of the stroma of the iris. They were large cysts filled with fluid, lined by pigmented epithelium and show a dense capillary network.

Zayed et al. (2012) reported that in buffalo histologically, the iris was covered anteriorly by a thin epithelial layer consisting of flat or fusiform cells underlined by a thin layer of spindle-shaped melanocytes (anterior stromal sheath) and posteriorly by a thick pigmented epithelium formed by highly folded layer of cuboidal to low columnar pigmented epithelial cells. The stroma of the iris was composed of a network of fine collagen fibers, which host blood vessels, iridal muscles and numerous melanocytes. Toward the pupillary border of the iris, the posterior pigmented epithelium demonstrated cystic-like collections forming the Corpora nigra.

2.7.1 Irido-corneal angle

Kassab et al. (2001) reported that the irido-corneal angle of buffalo included the pectinate ligament, the ciliary cleft, the trabecular meshwork (uveal and corneoscleral) and the angular aqueous plexus. The pectinate ligament was prominent anteriorly and a thick compact structure. The ciliary cleft was appeared quadrilateral and contained large amount of trabecular tissue that could be divided into two parts, the uveal part and the corneoscleral part. The uveal meshwork was composed of thick pigmented trabeculae in the anterior part, which became thin and loose in the posterior part. Their intertrabecular spaces were large and wide anteriorly. The corneoscleral meshwork was narrow anteriorly and widened posteriorly. Their trabeculae were non-pigmented and the intertrabecular spaces were small. The angular aqueous plexus consisted of four to five veins. They were located between the outer border of the corneoscleral meshwork and the inner border of the sclera.
Gelatt (2007) described that the irido-corneal angle (ICA) was formed by the junction of the corneoscleral tunic, base of the iris, and an anterior recession of the ciliary body, which was known as the cilio-scleral sinus or cleft. Pectinate ligaments span the opening of the cilio-scleral sinus from the pigmented corneoscleral junction to the root of the iris. Behind the pectinate ligaments and within the cilio-scleral sinus was a matrix of loose tissue strands, the trabecular meshwork. The trabecular meshwork consists of crisscrossing collagen cords that are covered by cells.

Kassab and Zoghby (2010) in goat reported that irido-corneal angel was comprised of the pectinate ligament, the ciliary cleft, the trabecular meshwork (uveal and corneoscleral) and the angular aqueous plexus.

2.8 RETINA

2.8.1 Histology

Prince et al. (1960) described the histology of retina in cattle and noted that it was composed of ten layers such as: (1) pigmented epithelium, (2) visual cell layer, (3) external limiting membrane, (4) outer nuclear layer, (5) outer plexiform layer, (6) inner nuclear layer, (7) inner plexiform layer, (8) ganglion cell layer, (9) optic nerve fiber layer, and (10) internal limiting membrane.

According to them, adjacent to the choroid there was a layer of flat polygonal cells which were more adhered to the choroid than to the visual cell layer. Pigmented epithelium was of little pigmented where it was backed by tapetum. The visual cell layer consisted of visual cells which were rods and cones. External limiting membrane was composed of the outer ends of fibers and the inner ends of which form the inner limiting membrane. The outer nuclear layer consisted of the nuclei of both the rods and cones. The outer plexiform layer was composed of the terminal arborizations of the rod and cones axon, and the dendrites of the bipolar cells of the
adjacent inner nuclear layer. The inner plexiform layer was also consisted of the arborization of the bipolar cells of the inner nuclear layer and ganglion cell layer on its another side. Layer of optic nerve fiber was formed by the fibers that leave the ganglion cell on their way to the optic nerve where they formed into bundles which become myelinated just after passing the lamina cribrosa. The internal limiting membrane was innermost layer of retina.

Dellmann (1993) mentioned the microscopic structure of the retina. Except at the transition towards the ora ciliaris retinae and at the optic disc, the retina was composed of ten layers as described earlier.

Khaled (2003) reported that histologically the bovine retina was consisted of ten layers such as (1) pigmented epithelium (2) layers of rods and cones (3) external limiting membrane (4) outer nuclear layer (5) outer plexiform layer (6) inner nuclear layer (7) inner plexiform layer (8) ganglion cell layer (9) optic nerve fiber layer (10) internal limiting membrane.

He reported that the retinal pigmented epithelium was a simple squamous or cuboidal epithelium resting on a basal lamina. The rod cells were long, slender and comprised an outer and inner segment. The rod nuclei contributed majority of nuclei of the outer nuclear layer. The cone cells also consisted of an outer and inner segment. The cone outer segment was a long conical structure, considerably wider than a rod at its base and tapering down to a blunt rounded tip. Proximal to the outer limiting membrane, the inner cone segment was found containing the nucleus that was larger and paler than the rod nucleus. The nuclei of the cones, in contrast to those of the rods were arranged in a single row immediately beneath the outer limiting membrane. The external limiting membrane separated the layer of rod and cone outer segments from the outer nuclear layer. The outer nuclear layer was composed mainly of rod’s and
cone’s nuclei arranged in 6 rows. The outer plexiform layer was a thin layer that separated the outer nuclear layer from the inner nuclear layer. It was composed mainly of the horizontal cell processes. The inner nuclear layer was thinner than the outer nuclear layer. The axons of the bipolar cells and the dendrites of the ganglion cells formed the inner plexiform layer which was a thick layer. The ganglion cell layer included the nuclei and cell bodies of the retinal ganglion cells of varying sizes, arranged in one or several layers. The ganglion cell bodies were very large and have round, eccentric nuclei and abundant cytoplasm. The non-mylinated axons of the ganglion cells were arranged parallel to the surface of the retina, forming a thick layer of the optic nerve fiber. The inner most layer of this nerve fiber layer was composed of the end feet of the supporting glial (Muller’s) cells. The inner limiting membrane lies between the vitreous body and the end feet of the supporting glial cells of the retina.

Gelatt (2007) described that the inner sensory retina contained nine layers, and the supportive pigmented epithelium which was the tenth layer. The optic retina extended from the optic disc to the ora ciliaris retinae, in which it was reduced to the two epithelial cell layers of the ciliary body. The retinal pigmented epithelium (RPE) was a layer of flat, polygonal cells that form the outermost part of the retina. The cell were usually densely pigmented but devoid of pigment in the dorsal choroid that contain the tapetum lucidum. The neurosensory layer varied in thickness, being thickest near the optic disc and tapering to the ora ciliaris retinae. The width of each layer decreased but the nerve fiber layer contributed the most to the variation in thickness. The visual cell layer contained only the outer and inner segment of the rods and cones with their nuclei in the outer nuclear layer. Outer nuclear layer contained the soma or cell bodies of the photoreceptors cell. The number of rows of nuclei varied greatly
according to the species and location in the retina. The outer nuclear layer gradually thin in the peripheral retina as the density of rods and cones decreased. Cone nuclei were universally situated next to the external limiting membrane. In mammals, cones nuclei were usually larger and oval than rod nuclei. The outer plexiform layer consisted of synapses between the rods and cones axons and dendrites of the bipolar and horizontal cells. Inner nuclear layer was consisted of the soma of the horizontal, bipolar, amacrine and Muller cells. The horizontal cell nuclei were positioned along the outermost margin of the inner nuclear layer, whereas the amacrine cell were positioned along the innermost margin. The bipolar nuclei and Muller cell nuclei compose the intermediate zone of the inner nuclear layer. The inner plexiform layer was notably thicker than the outer plexiform layer in all vertebrate animals especially those have fovea or well defined area centralis. Ganglion cell layer was the inner most layer of the retina and consisted of the single layer of cell, except in the area centralis and visual streak at which it might be two or three cell layer thick. Axon of ganglion cell gathered in the nerve layer, then turn at right angle and course to the posterior pole, at which the optic nerve exit. There were large retinal blood vessels in the nerve fiber layer as well as in the ganglion cell and inner plexiform layer. The nerve fiber layer was noted to be increased in thickness as it approached the optic disc.

2.8.2 Micrometry

Prince et al. (1960) in cattle mentioned that the total thickness of retina was about 220 µm, pigmented epithelium was about 10 µm thick, the outer nuclear layer was about 36 µm containing about 10 rows of outer nuclei. The inner nuclear layer was about 20 µm thick for 5-6 rows of inner nuclei.
Khaled (2003) in bovine reported the thickness of different layers of retina. Pigmented epithelium was about $9 \pm 0.9 \mu m$, External limiting membrane was $5 \pm 0.7 \mu m$, outer nuclear layer was $30 \pm 1.7 \mu m$, outer plexiform layer was $11 \pm 0.8 \mu m$, inner nuclear layer was $9 \pm 1.1 \mu m$ arranged in 3 rows, inner plexiform layer was $34 \pm 2.1 \mu m$, the ganglion cell layer was $12 \pm 0.6 \mu m$, a thick layer of optic nerve fiber was $25 \pm 1.4 \mu m$ and the inner limiting membrane was $6 \pm 0.3 \mu m$.

Gelatt (2007) mentioned that most animals had a central retina of approximately 200 to 240 $\mu m$ and a peripheral retina of 100 to 190 $\mu m$. In the central retina, the dog possessed the greatest depth of outer nuclear rows 12-15, whereas 10 rows in the cattle.

### 2.8.3 Artifacts of retina

Margo and Lee (1995) reported the role of fixative osmolarity in the production of tissue artifact in whole eye ball fixation. They reported that whole eyes fixed in 4% buffered formaldehyde (10% neutral buffered formalin) demonstrated a variety of artifacts, including separation of the neurosensory retina from the retinal pigment epithelium. They postulated that the osmolarity of 4% buffered formaldehyde causes contraction of the internal compartments of the eye leading to several artifactual changes commonly observed in routine histologic sections.

Chen and Nathans (2007) reported that in diverse mammals, multiple microscopic retinal folds or pseudorosettes were associated with some inherited retinopathies, foetal or early postnatal exposure to cytotoxic chemicals or ionizing radiation, and a variety of foetal or early postnatal viral infections.

Milles (2012) mentioned that folding of retina (Lang’s fold) usually occurred in eye balls of neonates and children fixed in formalin solution. This artifacts of fixation was not observed in the living eye or in unfixed eye. Lang’s fold was thought to be
resulted from traction on peripheral retina by shortening of the vitreous humor base and posterior lens zonules caused by tissue fixation. Retinal detachment was another retinal artifacts in which retina was detached from the Retinal Pigmented Epithelium.

2.9 **OPTIC NERVE**

Sisson and Grossman (1950) mentioned that the entrance of the optic nerve formed a sharply defined, oval and light area known as the optic papilla. It was situated about 15 mm ventral to the horizontal meridian and 3 to 4 mm lateral to the vertical meridian. The central part of the papilla was slightly depressed. The optic nerve fibers from all parts of the pars optica was converged to the papilla in which they were collected into bundles. These bundles traversed the lamina cribrosa of the choroidea and sclera, and constituted the optic nerve.

Bacha and Bacha (2000) mentioned that the optic nerve was formed by the nerve-fiber layer consisting of axonal processes of the ganglion cells that converged at the optic disc. Because the photoreceptor cells were not present here, this region was also referred to as the blind spot. Bundles of fibers of the optic nerve passed through perforations of the sclera. This sieve like part of the sclera was known as lamina cribrosa.

Samuelson (2007) mentioned that the hyaloid artery was providing nutrition to the lens during development in the fetus and was running forward to the lens from the optic disc. At birth the hyaloid artery was regressing, and was normally completely regressed by the time of eyelid opening. Sometimes, the hyaloid artery was not regressed completely and a remnant of hyaloid artery was found in the form of small papilla. This papilla was known as Bergmeister’s papilla and was frequently observed as an incidental clinical finding.
2.10 LENS

2.10.1 Biometry

Sisson and Grossman (1950) in domestic animals mentioned that crystalline lens was a biconvex and transparent body. It was situated in front of vitreous body and in partial contact with the posterior surface of the iris. The anterior surface was convex but the posterior surface was much more strongly curved than the anterior. The zonular ciliaris or suspensory ligament of the lens passed in a meridional direction from the ciliary processes to the capsule of the equator of the lens. The substance of the lens was enclosed by a highly elastic membrane known as the capsule of the lens.

Prince et al. (1960) mentioned that the crystalline lens was large with the steeper curvature on its posterior surface. They recorded the measurements of the different parameters of the lens in cattle as follow:

- Antero-posterior axis of the lens : 1.33 cm
- Diameter of the lens : 1.95 cm
- Thickness of Anterior capsule : 40.00 μm
- Thickness of Posterior capsule : 6.00 to 12.00 μm
- Thickness of Capsule at equator : 20.00μm

Martin and Anderson (1981) quoted the following measurements of various parameters of the lens in cattle as follow:

- Weight of the lens : 2.40 gm
- Antero-posterior axis of the lens : 1.20 cm
- Diameter of the lens : 1.72 cm
Panchbhai et al. (1988) recorded the biometry of the lens in buffalo calves. They recorded that the weight, antero-posterior axis and diameter of the lens were 1.56 gm, 1.004 cm and 1.47 to 1.84 cm respectively.

Banubakode (1992) reported the mean value of the different measurements of the lens in cattle as follow:

- **Weight of the lens**: $2.21 \pm 0.035$ gm
- **Thickness of the lens**: $1.22 \pm 0.008$ cm
- **Diameter of the lens**: $1.84 \pm 0.009$ cm

Gelatt (2007) mentioned that the lens was a transparent tissue without a direct blood supply in domestic animals. He noted the weight, average diameter and central thickness of the lens as 4.3 gm, 1.87 cm and 1.20 cm respectively in cow.

Khaled and Abdalla (2013) reported that the average weight of the lens was $2.44 \pm 0.23$ gm in buffalo.

### 2.10.2 Histology

Smythe (1956) reported that in domestic animals, the lens was covered by the capsule, below which there was a single layer of cuboidal epithelium.

Prince et al. (1960) mentioned that in domestic animals, the lens was mainly formed by the epithelial cells and completely covered by the capsule. Below the capsule there was a single layer of epithelial cells. These cells were cuboidal centrally and columnar towards the periphery. They recorded the measurements of the different parameters of the lens in cattle as follow:

- **Thickness of Anterior capsule**: $40.00 \, \mu$m
- **Thickness of Posterior capsule**: $6.00$ to $12.00 \, \mu$m
- **Thickness of Capsule at equator**: $20.00 \, \mu$m
Foster (1962) described the microscopic structure of the lens in human beings. He observed that the lens was surrounded by the capsule which was lined internally by a layer of cuboidal or columnar epithelial cells. He noted that the posterior surface of the lens was devoid of epithelial cells.

Martin and Anderson (1981) observed the histological structure of the lens in different species of animals. They stated that the lens was covered by capsule which was lined internally by the epithelium. Then they observed that the apical part of the cell was facing towards lens fibers whereas the basal part was towards the capsule. They found that the epithelium was cuboidal near the anterior pole but was columnar towards the equator.

Banubakode (1992) reported that the lens was composed mainly of three components such as the capsule, the epithelium and the lens fibers. The capsule was the outermost covering and homogeneous in appearance. A single layer of epithelial cells was found below the capsule. The epithelial cells were cuboidal at the center but towards the equator, they were elongated to form columnar cells. The lens fibers constituted the main body of the lens. The peripheral fibers were nucleated while the fibers in the center were devoid of nucleus.

Dellmann (1993) described microscopic structure of the lens in the cattle and found that lens was composed of lens capsule, lens epithelium and lens fibers. The lens was entirely surrounded by the lens capsule, which were made up of collagen fibrils arranged in several layers of lamellae. The rostral surface of the lens was lined by a simple cuboidal to columnar epithelium and at the equator, the epithelium differentiated into lens fibers. In the course of this process, the nuclei and most of the cells organelles were disappeared.
Gelatt (2007) noted that lens was completely enclosed within a thick, PAS positive elastic capsule. The thickness of the capsule varied by region to region, with the thinnest being the posterior pole. Inside the anterior capsule there was a single layer of lens epithelium. The cells were cuboidal to squamous, become columnar near the equator and elongated into slender hexagonal lens fibers. The lens epithelium lined only the anterior surface of the lens capsule and the equator postnatally.
MATERIALS
AND
METHODS
CHAPTER - 3

MATERIALS AND METHODS

The present study entitled “Gross and Histomorphological Study on Eye Ball of the adult Surti Buffalo (Bubalus bubalis)” was carried out at the Department of Veterinary Anatomy & Histology in collaboration with Department of Veterinary Surgery & Radiology and Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat. For this study, 20 eye specimens (10 right and 10 left) of buffaloes were used for the echobiometric, biometric and histological evaluation. The observations and results of the different parameters of the eye ball including cornea, sclera, choroid, ciliary body, iris, retina and lens of the adult surti buffalo were recorded in this study. The study was made irrespective of the sex of animals. All the samples were collected from healthy animals immediately after slaughtered in the local slaughter house.

The dissection of the eye balls was carried out by removing the extra ocular muscles, the fat and the peri-orbital tissue as much as possible in order to facilitate the correct measurement of the eye balls. The dorsal and ventral sides of the eye ball were determined by observing the tendon of dorsal oblique muscles and ventral exit of optic nerve respectively. The medial and lateral sides of the eye ball were also determined by finding the more pointed lateral end and the medial broader end of the cone shaped cornea. The measurements of the different parameters of the eye ball were recorded as follows:

3.1 Sonoanatomy and Echobiometrical dimensions of the eye balls

The ultrasonographic evaluation and ocular echobiometric dimensions of ten pairs of eye balls were done by using an ultrasound machine (e-saote MY Lab five
VET) using 7.5-18 MHz linear transducer (Fig. 1). The focal range 7.5-10 MHz was used for scanning of vitreous chamber and retinal wall and 12-18 MHz was used for the scanning of the anterior chamber and lens (Kumar, 2012).

Transcorneal approach was used and this technique involved placing the probe directly onto the cornea (Fig. 2).

3.1.3 Echobiometry parameters

The measurement of the different echobiometrical parameters of eye ball (Fig. 7) was done with the help of an ultrasound machine.

a) Anterior chamber depth
It was measured as the distance between the caudal wall of cornea and the anterior capsule of the lens.

b) Antero-posterior depth of Lens
It was measured as the distance between the anterior capsule and posterior capsule of the lens.

c) Vitreous chamber depth
It was measured as the horizontal distance between the posterior capsule of the lens and wall of retina.

d) Axial length of the globe
It was measured as the horizontal distance between the caudal wall of cornea and the wall of retina.

3.2 Biometry of the Eye Ball

The measurement of the different biometrical parameters of eye ball was done with the help of an electronic weighing balance and digital Vernier callipers (Banubakode, 1992).
a) **Weight**

Each eye ball was weighed on the scientific weighing balance in grams.

b) **Antero-posterior axis**

It was measured as the distance between the anterior and posterior pole of eye ball in centimeter with the help of digital Vernier callipers.

c) **Horizontal axis**

It was recorded as the distance between the entrances of the long posterior ciliary arteries on medial and lateral side of the eye ball (Fig. 3).

d) **Vertical axis**

It was recorded as the distance between the point of insertion of the dorsal oblique muscles and a point just ventral to the optic nerve.

### 3.3 Biometry of Cornea

a) **Horizontal diameter**

It was recorded as the longest distance between medial and lateral sclero-corneal junction i.e. limbus, with the help of digital Vernier callipers in centimeter.

b) **Vertical diameter**

It was measured as the maximum vertical diameter between the dorsal and ventral limbus with the help of digital Vernier calipers in centimeter.

c) **Thickness**

It was measured at the center and periphery of the cornea with the help of digital Vernier calipers in millimeter.
Fixation:

For the histological processing of cornea, sclera, choroid, ciliary body, iris and retina, the whole eye balls were fixed in Davidson’s fixative after giving an incision of about 10 cm to the limbus. The samples were then fixed for 24 - 30 hours.

Davidson’s fluid is an excellent fixative for fixation of whole eye ball but conventional 10% formalin causes artificial cellular shrinkage and poor cellular and nuclear resolution of the retina. Formaldehyde penetrate tissue well but takes more time because sclera stand as a physical barrier that protect the retina and inhibit the penetration of fixative (Shara et al., 2013). Davidson’s fixative is an acetic acid-alcohol-formalin based fixative in which alcohol denatured the protein by breaking hydrogen bonds and disturbing their tertiary structure and acetic acid increase the penetration. It has been advocated and widely used for the preservation of eye ball, maintaining retinal attachment during fixation and processing and providing better preservation of the retinal nuclear layer and sensory specialization of the rods and cones (Latendresse et al., 2002).

Composition of Davidson’s Fluid (Latendresse et al., 2002):

1) 39 % Formaldehyde : 2%
2) Ethanol : 35%
3) Glacial Acetic Acid : 10%
4) Distilled water : 53%

3.4 Biometry of Lens

After fixations, incision in the limbus was extended, then lens was extracted out and subjected for gross morphological study and biometry. Gentle pressure was given with the help of finger around the limbus with the object of breaking zonular
fibers. Due to zonulolysis, lens was extracted out. Then, the measurement of different parameters of the lens was recorded as follows:

a) **Weight**

Each lens was weighed on the scientific weighing balance in grams.

b) **Thickness**

The thickness of the lens was measured as the distance between the anterior and posterior poles of the lens with the help of the digital Vernier callipers.

c) **Diameter**

Diameter was recorded as the maximum distance between the points where two curvature meet each other in centimeter with the help of digital Vernier callipers (Fig. 4).

### 3.5. **Histology of Cornea, Sclera, Choroid, Ciliary body, Iris, Retina and Lens**

After fixation, the tissue pieces of the cornea and lens of 4-8 mm size and tunic (sclera, choroid and retina) of eye balls of 3-4 mm width were cut along with iris and ciliary body from the different regions because it was helpful to prevent detachment of retina from the choroid. They were kept in tap water for 60 to 90 minutes for sufficient removal of the fixative and treated with descending grades of alcohol and cleared in xylene. Then, the tissue were embedded in paraffin as per the method suggested by Drury and Wallington (1980). The paraffin blocks were sectioned at 5 - 7 µm thick.

The sections obtained were then subjected for routine and special staining techniques as under:

1. **H & E staining** (Singh and Sulochana, 1996).
2. **Masson’s trichome stain** for the collage fibers (Luna, 1968).
3. Periodic Acid Schiff (PAS) for demonstration of carbohydrate (Singh and Sulochana 1996).

3.6 Micrometry of Cornea, Sclera, Choroid, Retina and Lens

The micrometrical measurement were recorded with the help of graduated eye piece (Fig. 5 & 6) for the components of different tunics of the eye ball as per the method described by Culling (1969). The micrometry were taken at the center and periphery of each of the histological sections.

1. Total thickness of cornea in micron (µm).
2. Thickness of the epithelium of the cornea in micron (µm).
3. Number of layers of epithelial cells of cornea.
4. Thickness of the corneal stroma in micron (µm).
5. Thickness of the descemet’s membrane in micron (µm).
6. Thickness of endothelium of cornea in micron (µm).
7. Thickness of sclera, choroid and retina in micron (µm).

3.7 Statistical Analysis

The observations of echobiometry, biometry and micrometry were subjected to statistical analysis by using ‘t’ test as described by Snedecor and Cochran (1967) to find out the differences between the left and right eye balls and also among the individual eye balls.

The histological sections of cornea, sclera, choroid, ciliary body, iris, retina and lens were photomicrographed.
RESULTS
AND
DISCUSSION
CHAPTER - 4

RESULTS AND DISCUSSION

The present study entitled “Gross and Histomorphological Study on Eye Ball of the adult Surti Buffalo (Bubalus bubalis)” was carried out at the Department of Veterinary Anatomy & Histology in collaboration with Department of Veterinary Surgery & Radiology and Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat. For this study, 20 eye specimens (10 right and 10 left) of buffaloes were used for the echobiometric, biometric and histological evaluations. The study was made irrespective of the sex of animals. The observations and results of the different parameters of the eye ball including cornea, sclera, choroid, ciliary body, iris, retina and lens were recorded as follow:

4.1 ULTRASONOGRAPHY

4.1.1 Sonoanatomy

The eye balls of buffalo were appeared as an ovoid structures with mostly anechoic contents. The cornea appeared as a double-peaked echo (2 convex interfaces) with a central, narrow anechoic space. The aqueous chambers appeared as an anechoic space. The anterior and posterior lens capsule appeared as a convex and a concave echogenic line respectively and they were separated by the anechoic lens (Fig. 8).

The iris and ciliary body were seen as moderately echoic linear structure which extended from the peripheral globe towards the lens. The corpora nigra or iridica granules were seen as an echogenic tissue on the anterior surface of the dorsal
The iris was identified immediately adjacent to the anterior lens capsule with the thicker, irregular ciliary body lying peripheral to it. The vitreous chamber was appeared as a homogeneous, anechoic region between the posterior lens capsule and ciliary body anteriorly and the posterior ocular wall. Even though the posterior ocular wall had a good echogenicity, in this study, it was not possible to identify individual retinal, choroidal, or scleral layers (Fig. 8).

The double-peaked echo appearance of cornea was also reported by Assadnassab and Fartashvand (2013) in buffalo. However, in contrary to the present findings, the presence of corpora nigra or iridica granules was not mentioned by Spaulding (2008) in dog. The other general observations of sonoanatomy in surti buffalo were similar to those reported earlier by Whitcomb (2002) in horse, Spaulding (2008) in dogs and Assadnassab and Fartashvand (2013) in buffalo.

4.1.2 Echobiometry

The measurement of the different echobiometrical parameters of eye ball was done with the help of an ultrasound machine as follow (Fig. 7):

4.1.2.1 Anterior chamber depth

The overall mean value of the anterior chamber depth of the eye balls of both sides was 0.325±0.005 cm. The mean value of the anterior chamber depth in the right eye balls was 0.33±0.02 cm with the range between 0.25 to 0.45 cm and it was 0.32±0.01 cm with the range between 0.25 to 0.40 cm in the left eye balls.

The present observations are in agreement with the observations of Ribeiro et al. (2010) in male (0.346±0.055 cm) and female (0.333±0.046 cm) goats. However, Patil et al. (2011) in male horse reported that the right and left mean values were 3.96±0.13 mm and 4.22±0.17 mm respectively, which are found to be higher than surti buffaloes studied presently. Assadnassab and Fartashvand (2013) observed that
the right and left mean values were 0.291±0.014 cm and 0.287±0.015 cm respectively in buffalo which are comparatively lesser than that of surti buffaloes studied presently.

The observations of present study showed that the species variations are present in the anterior chamber depth of the eye balls. Horse was having the higher values and buffaloes was having the lower values and both are having more body weight. The less body weighing goat was having similar observation with the surti buffaloes studied presently. This means that the anterior chamber depth is not changing according to the weight of animals.

4.1.2.2 Antero-posterior depth of the lens

The overall mean value of the antero-posterior depth of the lens of both sides was 1.045±0.005 cm. The mean value of AP-depth of the lens in the right eye balls was 1.04±0.02 cm with the range between 0.89 to 1.10 cm and, it was 1.05±0.02 cm with the range between 0.90 to 1.10 cm in the left eye balls.

These observations are found to be lower than that reported by Patil et al. (2011) in male horse where the right and left mean values were 11.05±0.13 and 11.45±0.16 mm respectively and Assadnassab and Fartashvand (2013) in buffalo where the right and left mean values were 1.132±0.053 cm and 1.135±0.052 cm respectively. But, these observations are found to be higher than those reported by Ribeiro et al. (2010) in male and female goats where the mean values of antero-posterior depth of the lens were 0.860±0.034 and 0.865±0 cm respectively.

The present observations on antero-posterior depth of the lens showed that large animals like horse and buffaloes were having more depth than that of smaller animals like goat.
4.1.2.3 Vitreous chamber depth

The overall mean value of the vitreous chamber depth of the eye balls of both the sides was 1.635±0.005 cm. The mean value of vitreous chamber depth in the right eye balls was 1.63±0.05 cm with the range between 1.41 to 1.93 cm and, it was 1.64±0.05 cm with the range between 1.41 to 1.94 cm in the left eye balls.

The present observations are in agreement with the observations of Assadnassab and Fartashvand (2013) in buffalo where the right and the left mean values were 1.670 ± 0.040 cm and 1.677 ± 0.042 cm respectively. These observations are found to be lower than those reported by Patil et al. (2011) in male horse where the right and left mean values were 20.15±0.30 mm and 19.8±0.29 mm respectively. But, these observations are found to be higher than those reported by Ribeiro et al. (2010) in male and female goats where the mean values of vitreous chamber depth of the lens were 1.134±0.061 and 1.139±0.066 cm respectively.

The present study showed that species variations are present in the vitreous chamber depth of the eye ball. And also large animals such as buffalo and horse were having the higher values than the smaller animal like goat. This shows that the depth of the vitreous chamber is changing according to the size of animals.

4.1.2.4 Antero-posterior depth of the globe

The overall mean value of the antero-posterior depth of the globe of both sides was 3.135±0.005 cm. The mean value of the antero-posterior axis of the globe in the right eye balls was 3.13±0.05 cm with the range between 2.88 to 3.34 cm and, it was 3.14±0.05 cm with the range between 2.88 to 3.34 cm in the left eye balls.

The present observations are in agreement with the observations of Assadnassab and Fartashvand (2013) in buffalo where the mean values of right and
left were 3.292±0.037 cm and 3.297±0.037 cm respectively. However, the present observations are found to be lower than the observations of Patil et al. (2011) in male horse where the mean values of right and left were 35.28±0.34 mm and 35.50±0.32 mm respectively. Ribeiro et al. (2010) in male (2.343±0.092 cm) and female (2.339±0.086 cm) goats observed the lower values than the present observations.

The present study showed that species variations are present in the antero-posterior depth of the globe. Large animals such as horse and buffalo were having higher values than the smaller animals such as goat. So, the antero-posterior depth of the globe is changing according to the size of animals.

When comparing the different observations of the present study, the mean value of the anterior chamber depth of the eye ball in right side was found to be non significantly greater than that of the left side. The mean value of the vitreous chamber depth in right side was found to be similar with that of the left side. However, the mean values of antero-posterior depth of the lens and axial length of the globe on the left side were non significantly greater than that on the right side.

The statistical analysis showed non significant differences (p>0.05) between all the echobiometrical measurements of the right and left eye balls. Assadnassab and Fartashvand (2013) reported that the antero-posterior depth of the lens, vitreous chamber and axial length of the globe on the left side was greater than on the right but these differences were reported not to be statistically significant.

### 4.2 EYE BALL

The eye ball of surti buffalo was spherical in shape. The anterior surface was convex and the posterior surface was somewhat flattened (Fig. 9 & 13). The nictitating membrane was present at the medial side of the eye ball.
The eye ball was composed of three layers or coats. The outer coat was formed by the transparent membrane i.e. cornea, anteriorly and the white tough membrane i.e. sclera, posteriorly. The junction between the cornea and sclera known as limbus, was pigmented. The sclera was perforated by the optic nerve at the posterior part, lateral to the meridional plane and ventral to the horizontal plane. Two long posterior ciliary arteries were visible running towards the equator from the optic nerve in the superficial sclera (Fig. 10). Vortex veins were emerged from the sclera posterior to the equator of eye ball (Fig. 11). The sclera provided attachment to the tendons of extraocular muscles of the eye ball at the different surfaces.

The present observations are in accordance with the observations of Gelatt (2007) in domestic animals and Sisson and Grossman (1950) in cattle. The position of optic nerve, tendon of dorsal oblique muscle, nictitating membrane were very useful to identify the ventral, dorsal and medial sides of the eye balls respectively.

The present study revealed that the general features of the eye ball in other species were not different from those in the surti buffalo.

**4.2.1 Weight**

The overall mean value of the weight of the eye balls of both sides was 31.16±0.01 gm. The mean value of the weight of eye ball in the right eye was 31.15±0.85 gm with the range between 27.00 to 35.30 gm whereas, it was 31.17±0.76 gm with the range between 27.50 to 35.40 gm in the left eye balls.

The present observations are in agreement with the observations of Martin and Anderson (1981) in exotic cattle (32.50 gm). However, the present observations of eye balls are higher than those reported by Panchbhai *et al.* (1988) in buffalo calves (21.41 gm) and Banubakode (1992) in cattle (24.17±0.48 gm). Though, Khaled and
Abdalla (2013) reported the higher mean values of the weight in buffalo (35.30±2.69 gm) than the present observations.

The present study showed that there are variations not only between the species but also within the species as well. It also showed that the weight of the eyeball was changing with the age of animals.

### 4.2.2 Antero-posterior axis

The overall mean value of the antero-posterior axis of the eyeballs of both sides was 3.67±0.00 cm. The mean value of antero-posterior axis in both right and left eye balls was 3.67±0.04 cm each with the ranges between 3.40 to 3.85 cm in the right eye balls and 3.35 to 3.85 cm in the left eye balls.

The present observations are in agreement with the observations of Smythe (1956) in ox (3.53 cm), Martin and Anderson (1981) in exotic cattle (3.53 cm), Prince et al. (1960) in exotic cattle (3.40 to 3.70 cm) and Gelatt (2007) in cow (3.53 cm). However, the present observations were found to be higher than the observations of Panchbhai et al. (1988) in buffalo calves (3.19 cm) and Banubakode (1992) in cattle (3.27 ± 0.02 cm).

The present study showed that species variations are present in the antero-posterior axis of eye balls. The antero-posterior axis of eye balls was also changing according to the age of animals and breed of the animals (indigenous or exogenous).

### 4.2.3 Horizontal axis

The overall mean value of the horizontal axis of the eye balls of both sides was 4.04±0.00 cm. The mean value of the horizontal axis in both right and left eye balls was 4.04±0.03 cm each with the ranges between 3.90 to 4.27 cm in the right eye balls and 3.90 to 4.30 cm in the left eye balls.
The present observations are in agreement with the observations of Smythe (1956) in exotic cattle (4.19 cm), Martin and Anderson (1981) in exotic cattle (4.19 cm), Prince et al. (1960) in cow (3.80 to 4.30 cm) and Gelatt (2007) in cow (4.19 cm). However, Panchbhai et al. (1988) in buffalo calves (3.37 cm) and Banubakode (1992) in cattle (3.55 ± 0.041 cm) recorded the lower values than the present observations.

The present study showed that there are species variations in the horizontal axis of the eye balls. It also showed that the horizontal axis of the eye balls is changing according to the age of the animals.

4.2.4 Vertical axis

The overall mean value of the vertical axis of the eye balls of both sides was 4.01±0.01 cm. The mean value of the vertical axis in the right eye balls was 4.02±0.04 cm with the range of 3.80 to 4.36 cm and, it was 4.00±0.02 cm with the range of 3.90 to 4.10 cm in the left eye balls.

The present observations are in agreement with the observations of Martin and Anderson (1981) in exotic cattle (4.08 cm), Prince et al. (1960) in exotic cattle (3.70 cm to 4.20 cm), Smythe (1956) in ox (4.08 cm) and Gelatt (2007) in cow (4.082 cm). However, the present observations are found to be higher than the observations of Panchbhai et al. (1988) in buffalo calves (3.45 cm) and Banubakode (1992) in cattle (3.23±0.08 cm).

The present study showed that there are species variation in the vertical axis of the eye balls. It also showed that the vertical axis of the eye balls is changing according to the age of the animals.

There were slight differences between the biometrical observations of the right and left eye balls in the present study. The vertical axis was found to be lower than the horizontal axis of the eye balls in surti buffaloes. This observation is in agreement
with all the observations in other animals. The differences in the biometrical mean values of the eye balls among the animals may be due to differences in the age of animals or the breed variation or the species variation. In general, the body size of the exotic buffalo or cattle is larger than that of the indigenous buffalo and hence, the lower biometrical observation of the eye balls in indigenous buffalo may be observed.

4.3 CORNEA

Grossly, the cornea was found to be an elliptical transparent membrane having a broader end at the medial side and a more pointed end at the lateral side. Almost all the anterior visible portion of the eye ball was covered by it (Fig. 9). The anterior surface was convex while the posterior surface was concave (Fig. 12). Similar observations were reported earlier by Sisson and Grossman (1950) in domestic animals, Gelatt (2007) in cow and Ghosh (2012) in cattle.

The present study showed that there was no variation in the general structures of cornea among the different domestic animals.

4.3.1 Biometry

4.3.1.1 Vertical Diameter

The overall mean value of the vertical diameter of the cornea of both the sides was 2.37±0.00 cm. The mean value of the vertical diameter of cornea was 2.37±0.03 cm in right eye balls with the range between 2.20 to 2.50 cm and it was 2.37±0.02 cm with the range between 2.28 to 2.50 cm in left eye balls.

The present observations are in agreement with the observations of Smythe (1956) in ox (2.40 cm), Prince et al. (1960) in exotic cattle (2.20 to 2.40 cm) and Martin and Anderson (1981) in exotic cattle (2.35 cm). The present values are found to be lower than those reported by Banubakode (1992) in cattle (2.42±0.02 cm).
Whereas, the values are found to be higher than those reported by Panchbhai et al. (1988) in buffalo calves (2.05 cm) and Faber et al. (2008) in pig (1.24 cm).

The present study showed that there were species variations in the vertical diameter of cornea. The large animals such as buffalo and cattle were having higher values of vertical diameter of cornea than the small animals such as pig. This showed that the value of vertical diameter was changing according to the size of animals. The study also showed that the value of vertical diameter was changing according to the age of animals in buffalo.

4.3.1.2 Horizontal Diameter

The overall mean value of the horizontal diameter of the cornea of both sides was 2.905±0.015 cm. The mean value of the horizontal diameter of cornea was 2.89±0.04 cm in right eye balls and 2.92±0.04 cm in left eye balls with the range between 2.70 to 3.20 cm each in both right and left eye balls.

The present observations are in agreement with the observations of Smythe (1956) in exotic cattle (2.9 cm) and Prince et al. (1960) in cattle (2.70 to 3.20 cm). Martin and Anderson (1981) in exotic cattle (3.05 cm) and Banubakode (1992) in cattle (3.25±0.03 cm) reported the higher values than the present observations. However, Panchbhai et al. (1988) in buffalo calves (2.53 cm) and Faber et al. (2008) in pig (1.49 cm) reported the lower value than the present observations.

The present study showed that the horizontal diameter of the cornea was changing according to the species, size and age of animals.

4.3.1.3 Thickness

The overall mean value of the thickness of cornea was 1.20±0.09 mm. The mean value of the thickness was 1.11±0.08 mm at the center of cornea and 1.29±1.29
at the periphery of cornea with the range between 0.85 to 1.50 mm at the center and 0.90 to 2.00 at the periphery.

The present study showed that species variations are present in the thickness of cornea. The present observations are found to be lower than the observations of Gelatt (2007) in cow where the thickness of cornea at center and at edge were 1.5-2.0 mm and 1.5-1.8 mm respectively. However, the present observations are found to be higher than the observations of Faber et al. (2008) in pig where the observations were 0.666 mm centrally and 0.714 mm peripherally.

The present study showed that the thickness of cornea was more in the periphery than the center of cornea which is in agreement with the findings of Faber et al. (2008) in pig whereas the observation is in contrary to the findings of Gelatt (2007) in cow.

4.3.1.4 Ratio between vertical and horizontal diameters

The overall mean ratio of vertical diameter to horizontal diameter of both the sides was 0.816. This value showed that the horizontal diameter of cornea was higher than the vertical diameter of cornea. This observation is in agreement with the observations of other domestic animals.

The ratio of the height to the width of the cornea gives us an idea about the shape of cornea. This ratio was found to be more or less similar to other observations reported in cattle and buffalo calves. This variation in the shape of the cornea may be due to the difference in the habits as opined by Prince et al. (1960). According to them, the variation in the shape of cornea occurred as the needs demanded by the animals.

4.3.2 Histology
In the present study, the cornea was observed to be composed of four layers (Fig. 16). This finding is similar to that noted by Martin and Anderson (1981) in domestic animals excluding cattle and Ramkrishna et al. (1997a) in Indian water buffalo. The four layers from the outward to the inward were as below:

1. Anterior Epithelium
2. Corneal stroma / Substantia Propria
3. Descemet’s Membrane and
4. Endothelium

4.3.2.1 Anterior epithelium

It was the outermost layer of the cornea and was composed of rows of epithelial cells. These cells were of stratified squamous non-keratinized type and the nuclei of these epithelial cells were of blackish blue coloured in H&E staining (Fig. 17). The basal cells of the epithelial layers were resting on the basement membrane and were of columnar type while the cells towards the anterior surface were found to be progressively flattened or squamous type (Fig. 17 & 19).

4.3.2.2 Corneal stroma or substantia propria

This layer constituted the major part of the cornea and was found to be composed of regularly arranged sheets or lamellae of the collagen fibers along with the fibroblast cells. The nuclei of fibroblast cells were easily recognized from their elongated shape along the fibers (Fig. 17)

4.3.2.3 Descemet’s membrane

This membrane was found to be interposed in between the stroma and the endothelium. It was uniformly thick, homogenous, eosinophilic membrane. It was composed of loosely arranged collagen fibers which were faintly stained (Fig. 18)

4.3.2.4 Endothelium
The endothelium was found to be the last and the most posterior layer of the cornea. It was composed of a single row of flattened cells with prominent elongated nuclei lying at the caudal border of Descemet’s membrane parallel to the surface of cornea (Fig. 18).

The present observations of the different layers of the cornea such as the anterior epithelium, corneal stroma, Descemet’s membrane and endothelium in surti buffalo are in agreement with the observations of Prince et al. (1960) in different domestic animals, Martin and Anderson (1981) in cattle, Ross and Edward (1985) in human beings, Banubakode (1992) in cattle, Ramkrishna et al. (1997a) in Indian water buffalo, Khaled (2003) in bovine and Gelatt (2007) in domestic animals.

The present study showed that the general histological structures of cornea in different species of animals were similar to one another. However, in contrary to the present findings, the layer of the cornea i.e. “Bowman’s membrane” reported by Smythe (1956) and Prince et al. (1960) in cattle, Ross and Edward (1985) in human beings, Martin and Anderson (1981) in cattle, Banubakode (1992) in cattle and Khaled (2003) in bovine eyeball was indistinct in the present study. Gelatt (2007) had mentioned the absence of Bowman’s layer in most animals. Ramkrishna et al. (1997a) also reported that the subepithelial basal lamina was not evident in Indian water buffalo.

4.3.3 Micrometry

4.3.3.1 Thickness of the epithelium

The overall mean value of the thickness of the epithelium of both the center and periphery of cornea was 99.29±0.625 µm. The mean value of the thickness of the epithelial layer was 99.92±5.32 µm with the range between 67.59 µm to 132.00 µm at
the periphery and it was 98.67±4.49 μm with the range between 46.20 μm to 121.20 μm at the center.

The present observations are in agreement with the observations of Khaled (2003) in bovine (98.00 ± 1.50 μ). The observations of the thickness of epithelial layer of Prince et al. (1960) in exotic cattle (90 μm), Bloom and Fawcett (1962) in human beings (50 μm) and Banubakode (1992) in cattle (72.08±2.90 μm) were lower than those of the present observations.

4.3.3.2 Number of epithelial cell layers

The overall mean value of the number of the epithelial layers of both the center and peripheral cornea was 9.35±0.06. The average number of the epithelial layers of the cornea was 9.42±0.11 with the range between 7 to 12 cells at the periphery and it was 9.29±0.11 with the range between 7 to 12 at the center.

The present observations are in corroboration with the observations of Banubakode (1992) in cattle (9.28±0.17 layers). However, the observations are found to be much lower than the observations of Diesem (1977) in bovines (14 to 18 rows) and Camber et al. (1987) in pigs (17 to 23 rows). The average number of epithelial layers was more in the periphery than at the center of cornea which is similar to the findings of Martin and Anderson (1981).

4.3.3.3 Thickness of corneal stroma

The overall mean value of the thickness of stroma of both the center and periphery of cornea was 662.45±4.86 μm. The mean value of the thickness of the corneal stroma was recorded as 667.32±67.27 μm with the range of 324.24 μm to 1080.80 μm at the periphery and it was 657.59±63.05 μm with the range of 330.21 to 996.66 μm at the center.
The present observations in surti buffaloes are found to be higher than the observations of Khaled (2003) in bovine, who reported the mean value of the thickness of corneal stroma as 580±40 μm.

**4.3.3.4 Thickness of Descemet’s membrane**

The overall mean value of the thickness of the Descemet’s membrane of the center and periphery of cornea was 18.705±0.08 μm. The mean value of the thickness of the Descemet’s membrane was 18.79±2.36 μm with the range of 9.90 μm to 27.02 μm at the periphery and it was 18.62±2.31 μm with the range between 9.9 to 26.42 μm at the center.

The present observations are found to be higher than the observations of Prince *et al.* (1960) in cattle (10 to 25 μm) and Bloom and Fawcett (1962) in human beings (5 to 10 μm). However, the present observations are found to be lower than the observation of Khaled (2003) in bovine (30±1.0 μm).

**4.3.3.5 Thickness of the endothelium**

The overall mean value of the thickness of endothelium of both the center and periphery of cornea was 5.015±1.83 μm. The mean value of the thickness of endothelium was 5.08±0.37 μm with the range between 3.3 to 6.6 μm at the periphery and it was 4.95±0.35 μm with the range between 3.3 to 6.6 μm at the center.

The present observations are lower than the observations of Prince *et al.* (1960) in cattle (6 μm), Diesem (1977) in bovines (6 μm) and Khaled (2003) in bovine (8±0.3 μm). Banubakode (1992) in cattle observed the thickness of endothelium as 2.22±0.067 μm which is around half of the present observations.

**4.3.3.6 Total thickness of the cornea**

The overall mean value of the total thickness of both the center and periphery of the cornea was 794.05±4.92 μm. The mean value of the thickness of the cornea
was 798.607±69.17 µm with the range between 378.28 µm to 1215.90 µm at the periphery and it was 789.13±64.20 µm with the range between 378.28 to 1109.93 µm at the center.

The present observations are in agreement with the observations of Prince et al. (1960) in cattle (750 to 850 µm) and Bloom and Fawcett (1962) in human beings (800 to 900 µm). However, the present observations are found to be lower than the observations of Diesem (1977) in bovines (1500 to 2000 µm) and Banubakode (1992) in cattle (933.72 ± 15.35 µm), whereas, the observations are found to be higher than the observations of Camber et al. (1987) in pig (722 µm).

The present study clearly showed that variations are present in the thickness of epithelium, the number of cell layers in the epithelium, the thickness of stroma, the thickness of Descemet’s membrane, the thickness of endothelium and the total thickness of cornea in different animals. These variations in the different observations of cornea may be due to the differences in the species, breed or some other factors.

In the present study, the thickness of the different layers of cornea was found to be varied from region to region. The mean value of the thickness of cornea was the thickest at around the limbus and the lowest in between the center and the limbus. However, the observations of the cornea showed non significant differences (p>0.05) between the center and the periphery.

4.4 SCLERA

Grossly, the sclera was found to be a white tough membrane located behind the cornea at the posterior portion of the fibrous tunic. The thickness of sclera was found to be varied from region to region, the thickest at around the optic nerve and the thinnest at the equator (Fig. 13). The present observations are found to be similar with the observations of Sisson and Grossman (1950) in domestic animals.
4.4.1 Histology

The sclera was found to be composed of collagenous bundles running in various directions with fibroblasts and occasional melanocytes between them. It can be subdivided into three layers as follow (Fig. 20A):

(1) The outermost layer or the episclera
(2) The middle layer or the sclera proper
(3) The innermost layer or lamina fusca

The episclera was found to be loose and vascular having fibrous connective tissue.

The sclera proper was composed of bundles of collagenous fibers which were oriented mainly parallel to the surface but with some interweaving. Elongated fibroblasts and melanocytes were found in some areas of the bundles of collagen fibers. Adjacent to the choroid, the innermost layer or lamina fusca or dark layer was present. This layer was composed of much smaller bundles of collagenous fibers with numerous melanocytes.

Similar observations were reported earlier by Dellmann (1993) in domestic animals, Ramkrishna et al. (1997b) in Indian water buffalo and Khaled (2003) in bovine eye ball. The present study showed that the general features of sclera are similar in the different domestic animals.

4.4.2 Micrometry

The histological mean values of the thickness of the sclera at the periphery was 445.96±23.05 µm with the range between 297.22 to 540.33 µm and that of the center was 856.95±33.84 µm with the range between 743.05 to 1080.80 µm.

Khaled (2003) in bovine eye ball reported that the thickness of sclera was 1132±24.4 µm, which is found to be much higher than the present observations. This
variation in the thickness of sclera may be due to the differences in the species, breed or some other factors.

4.5 CHOROID, CILIARY BODY AND IRIS

4.5.1 CHOROID

Grossly, the choroid was observed to be a dark pigmented membrane, located between sclera and retina. Anteriorly it was continued as ciliary body and iris. The greenish or bluish coloured membrane, known as tepetum lucidum was found at the dorsal half of the choroid. Similar observations were reported earlier by Sisson and Grossman (1950) in domestic animals and Ghosh (2012) in cattle.

4.5.1.1 Histology

The choroid was found to be composed of four layers such as

1. Suprachoroid
2. Large vessel layer / vascular layer
3. Tapetum and
4. Small vessel layer / choriocapillary layer

In some area of the choroid mainly ventral to the optic nerve, the tapetum was found to be absent.

The suprachoroid layer was composed of the collagen fibers along with pigmented connective tissue that formed a transition between the sclera and the choroid. The vascular layer was composed of large and medium sized blood vessels embedded in loose connective tissue containing melanocytes and fibrocytes. The tapetum layer was fibro-vascular consisting of intermingling collagen fibers and few fibroblast and the arterioles surrounded by pigment cells were seen in this layer. The fourth layer, choriocapillary layer, was composed of a single layer of capillaries next
to the tapetum and also the capillaries were arranged in a continuous chain form (Fig. 30, 32 and 33).

These observations are in accordance with the observations of Prince et al. (1960) in cattle, Dellmann (1993) in domestic animals, Ramkrishna et al. (1997) in Indian water buffalo, Khaled (2003) in bovine and Gelatt (2007) in domestic animals. However, in contrary to the present observations, they all reported the presence of Bruch’s membrane which was difficult to identify in the present study.

4.5.1.2 Micrometry

The mean thickness of the choroid was 76.55±3.72 µm with the range between 66.00 µm to 94.57 µm in the center of the choroid whereas, it was 48.86±1.78 µm with the range between 40.53 to 59.63 µm in the peripheral section of choroid. The thickness of tapetum lucidum was varied from 16.5 to 40.53 µm with the average of 29.10 µm.

Prince et al. (1960) in cattle mentioned that the thickness of the choroid was about 100 to 160 µm excluding a tapetum. The thickness of tapetum was from 10 µm at the periphery to 50 µm at the center. Khaled (2003) in bovine reported the thickness of choroid as 208.6±6.905 µm. The present observations of the thickness of choroid are much lower than these observations but for the tapetum lucidum, it was in accordance to their observations. These variations in the thickness of choroid may be due to the differences in the species, breed or some other factors.

4.5.2 CILIARY BODY

In the present study of adult surti buffalo, the ciliary body was found to be composed of ciliary muscles, collagen fibers, blood vessels and processes (Fig. 24). The smooth muscle fibers were parallel to sclera with few circular fibers. The cell population of ciliary body was composed of fibroblasts, numerous flat melanocytes,
some smooth muscle cells. The basal plate presented connective tissue core with large blood vessels, arteries and veins and this layer extended as dense network of capillaries into the ciliary processes. The ciliary processes were composed of numerous thin folds covered by two layers of stratified cuboidal epithelium, whose superficial cells were non pigmented while the deeper cells were deeply pigmented (Fig. 25).

The present observations are similar with the observations of Ramkrishna et al. (1997b) in Indian water buffalo and Khaled (2003) in bovine.

4.5.3 IRIS

Grossly, the iris was found to form a dumb bell shaped pupil in the present study. At the pupillary margin, some black granules like structures were found, which were more prominent in the dorsal part. These structures were known as iris granules (Fig. 8). The similar observations are also reported earlier by Sisson and Grossman (1950) in domestic animals.

Histologically, the thickness of the iris was observed to be decreased towards the free margin (Fig. 26). The stroma was composed of loose connective tissue with smooth muscles, blood vessels, melanocytes and fibroblasts. The anterior surface was composed of pigmented cells and the posterior surface showed two layered pigmented epithelium (Fig. 27).

The present histological observations of iris in adult surti buffalo are in accordance with the observations of Khaled (2003) in bovine, Ramkrishna et al. (1997b) in Indian water buffalo and Zayed et al. (2012) in buffaloes.

4.5.3.1 Irido-corneal angle

The irido-corneal angle was the area located at the periphery of the anterior chamber. The irido-corneal angle was formed by the junction of the corneoscleral
tunic (Limbic zone), base of the iris, and anterior ciliary body (Fig. 20B). The iridocorneal angle was comprised of the pectinate ligament, the ciliary cleft, the trabecular meshwork (uveal and corneoscleral) and the angular aqueous plexus (AAP).

The pectinate ligament was located on the anterior part of the iridocorneal angle. It was a strong, band-like structure extending from the iridal base to the limbic zone. The ciliary cleft was the space, which was bordered by the pectinate ligament anteriorly, the limbal zone from the outer aspect, and the base of the iris and the ciliary body from the inner aspect. The ciliary cleft was containing large amount of trabecular tissue. Trabecular tissue had two parts: the uveal part and the corneoscleral part. The uveal meshwork was the inner part of the trabecular meshwork. It was composed of numerous strands of trabeculae. The corneoscleral meshwork was the external part of the trabecular meshwork. The trabeculae were made of collagen, and there were melanin pigments and endothelial cells on their surface. There were intertrabecular spaces between the trabeculae. These spaces were wide anteriorly and decreased gradually till became narrow posteriorly. The AAP consisted few veins lined by endothelial cells positioned between the outer border of the corneoscleral meshwork and the inner border of the sclera.

The observations of present study are similar as described by Kassab et al. (2001) in buffalo, Gelatt (2007) in domestic animal and Kassab and Zoghby (2010) in goat.

4.6 RETINA

Retina was located in the innermost layer of the tunics of eye ball (Fig. 28). It was a very thin, delicate membrane with many blood vessels.

4.6.1 Histology
The retina can be divided into two parts, the part which covered the posterior portion of vascular tunic i.e. choroid known as pars optica retinae and the part which covered the anterior portion of vascular tunic i.e. ciliary body and iris known as pars ciliaris retinae and pars iridae retinae respectively. The junction between the two parts is known as ora ciliaris retinae (Fig. 34).

The retina of adult surti buffalo was found to be composed of ten layers which were from outside to inside as follow (Fig. 29):

1. Pigmented epithelium
2. Layer of rods and cones
3. External limiting membrane
4. Outer nuclear layer
5. Outer plexiform layer
6. Inner nuclear layer
7. Inner plexiform layer
8. Ganglion cell layer
9. Nerve fibers layer
10. Internal limiting membrane.


Pigmented epithelium: The pigmented epithelium was simple cuboidal or squamous epithelium with prominent nuclei. It was deeply pigmented but was of little pigmented where it was backed by tapetum.
Layer of rods and cones: The next layer was the layer of rod and cone cells situated between the layers of pigmented epithelium and the external limiting membrane.

External limiting membrane: The external limiting membrane was a thin membrane and found to be situated between the layer of rods and cones and the layer of outer nuclei.

Outer nuclear layer: The layer of outer nuclei was composed of the nuclei of both rods and cones arranged in layers of about 7 to 9 rows.

Outer plexiform layer: The outer plexiform layer was situated between the outer nuclear layer and the inner nuclear layer and was observed to be thinner than the inner plexiform layer.

Inner nuclear layer: The inner nuclear layer was composed of around 3-4 rows of nuclei and it was found to be thinner than the outer nuclear layer.

Inner plexiform layer: The inner plexiform layer was situated between the inner nuclear layer and the ganglion cell layer.

Ganglion cell layer: The ganglion cells were usually arranged in a single row and are found to be absent where retinal blood vessels were present.

Nerve fibers layer: The nerve fibers layer was present between the ganglion cell layer and the internal limiting membrane. The presence of retinal blood vessels in this part of retina increased the thickness of the retina. The thickness of nerve fiber layer was found to be increased near the optic disc (Fig. 31).

Internal limiting membrane: The internal limiting membrane was the innermost thin membrane and was in contact with the vitreous body.
The present observations of general histological structures of retina of adult surti buffalo are found to be in agreement with the observations of Prince et al. (1960) in domestic animals, Khaled (2003) in bovine and Gelatt (2007) in domestic animals.

4.6.2 Micrometry

The mean thickness of the retina in the center of the tunics was 177.56±10.72 µm with the range from 155.10 µm to 229.67 µm and that of retina in the peripheral section of the tunics was 120.24±15.40 µm with the range from 94.57 µm to 149.33 µm. The present observations are lower as compared to the observations of Prince et al. (1960) in cattle (220 µm) and Gelatt (2007) in domestic animals (central retina of 200 to 240 µm and peripheral retina of 100 to 190 µm). However, the individual thickness of retina near to the optic nerve was as high as 270.20 µm in the present study.

The mean thickness of the different layers of retina were as below

(a) Pigmented epithelium  5.36±0.35 µm (range between 3.30 to 6.60 µm)
(b) Layer of rods and cones  25.30±0.74 µm (range between 23.10 to 29.70 µm)
(c) Outer nuclear layer  35.20±0.74 µm (range between 30.00 to 39.60 µm)
(d) Outer plexiform layer  6.05±0.23 µm (range between 4.95 to 6.60 µm)
(e) Inner nuclear layer  22.82±0.63 µm (range between 19.80 to 26.40 µm)
(f) Inner plexiform layer  22.27±0.91 µm (range between 16.50 to 26.40 µm)

The average number of rows of nuclei in the outer and inner nuclear layer were 8.16±0.20 and 3.3±0.14 rows respectively.

Prince et al. (1960) in cattle and Khaled (2003) in bovine reported the thickness of pigmented epithelium as about 10 µm and 9 ± 0.9 µm respectively. These values are higher than the mean value of the present observation i.e. 5.36±0.35 µm.
Prince et al. (1960) in cattle mentioned the thickness of outer nuclear layer as 36 µm. This value is in accordance with the present mean value of the outer nuclear layer i.e. 35.20±0.74 µm. However, Khaled (2003) in bovine reported that the mean value of the outer nuclear layer was 30 ± 1.70 µm which was slightly lower than that of the present observation.

The present observation of thickness of inner nuclear layer (22.82±0.63 µm) is in agreement with the observation of Prince et al. (1960) in cattle (20 µm). However, the present observation is found to be higher than the observation of Khaled (2003) in bovine (9 ± 1.10 µm).

The present observation of the thickness of outer plexiform layer (6.05±0.23 µm) is found to be lower than the observation of Khaled (2003) in bovine (11 ± 0.80 µm).

Khaled (2003) in bovine reported the thickness of inner plexiform layer as 34 ± 2.10 µm. In the present study, the thickness of inner plexiform layer was 22.27±0.91 µm which was lower than the observation of Khaled (2003) in bovine.

Prince et al. (1960) in cattle mentioned the presence of around 10 rows of nuclei and Khaled (2003) in bovine reported the presence of around 6 rows of nuclei in the outer nuclear layer. The present mean value of the number of nuclei in outer nuclear layer was 8.16±0.20 which was found to be lesser than the observation of Prince et al. (1960) in cattle but higher than the observation of Khaled (2003) in bovine.

The present mean value of the number of nuclei in the inner nuclear layer was 3.3±0.14 which is found to be in accordance with the observation of Khaled (2003) in bovine, where he reported the nuclei of inner nuclear layer was arranged in 3 rows. Prince et al. (1960) in cattle mentioned the presence of 5-6 rows of inner nuclei in the
inner nuclear layer which is found to be slightly higher than those of the present observation.

The variations in the values of thickness of different layers of retina may be due to variations in the species of animals or some other factors.

4.6.3 Artifacts of retina

The present study showed that retina detached from the retinal pigmented epithelium and sometimes retina also detached from the choroid. At the site of retinal detachment, outer segment of the intact photoreceptors and fragment of retinal pigment epithelium were present. Similar findings are reported by Margo and Lee (1995) and Milles (2012).

In more than 60-70 % of the samples, it was found that retina took a concave appearance vitreally which was looking like a fold (Fig. 35). It was almost round and oval in shape. The retinal fold was not observed in unfixed enucleated eye. This retinal fold may be due to either artifacts of fixation as earlier described by Milles (2012) or hereditary as reported by Chen and Nathans (2007). The fixative (formalin) causes the shrinkage of fibrous tunic, shortening of the vitreous body and posterior lens zonules that leads to traction of retina and resulting into folding of very delicate and soft layer of nervous tissue i.e. retina. These findings are in accordance with the observations of Margo and Lee (1995) and Milles (2012).

4.7 OPTIC NERVE

Histologically, the convergence of nerve fibers at the optic disc to form optic nerve was observed. The nerve fibers then passed through the sieve like structure of sclera known as lamina cribrosa (Fig. 36). Similar observations were reported earlier by Sisson and Grossman (1950) in domestic animals and Bacha and Bacha (2000) in dog.
A small papilla, projected towards the vitreous cavity from the optic disc, was found in some histological sections. This papilla is a remnant of hyaloid artery, which supplied nutrition to lens during development in the fetus and is called as Bergmeister’s papilla (Fig. 37). Similar observation was reported earlier by Samuelson (2007).

4.8 LENS

Grossly, the lens was found to be transparent (Fig. 14), biconvex and soft substance located in front of the vitreous body. The convexity was more in the posterior part and it was somewhat flattened in the anterior part. It was completely enclosed within a very thin capsule called anterior capsule and posterior capsule. The zonulary fibers were found to be attached to the equatorial portion of the lens holding the lens in proper position (Fig. 15). Similar observations were also mentioned earlier by Sisson and Grossman (1950) in domestic animals.

4.8.1 Biometry

4.8.1.1 Weight

The overall mean value of the weight of the lens of both the sides was 2.525±0.005 gm. The mean value of the weight was 2.52±0.07 gm with the range between 2.29 to 2.83 gm in right eye balls and, it was 2.53±0.07 gm in left eye balls with the range between 2.30 to 2.90 gm.

The present observations are in accordance with the previous observations of Martin and Anderson (1981) in exotic cattle (2.4 gm) and Khaled and Abdalla (2013) in buffalo (2.44 ± 0.23 gm). But the present observations are found to be higher than those reported by Panchbhai et al. (1988) in buffalo calves (1.56 gm) and Banubakode (1992) in cattle (2.21±0.035 gm) and found to be lower than that reported by Gelatt (2007) in cow (4.3 gm). Bosch et al. (1983) stated that the weight of the lens increases
as the age of the animal advances, so the present value in adult surti buffalo was seemed to be much higher than the buffalo calves. The difference in the weight of the lens may also be due to species variation.

4.8.1.2 Thickness/Antero-posterior axis

The overall mean value of the thickness of the lens of both the sides was 1.32±0.00 cm. The mean value of the thickness in both right and left eye balls was 1.32±0.01 cm each with the range between 1.29 to 1.42 cm in right eye balls and 1.28 to 1.42 cm in left eye balls.

The present observations are in concurrence with the observations of Prince et al. (1960) in cattle (1.33 cm). These observations are found to be higher than those of Martin and Anderson (1981) in exotic cattle (1.20 cm), Panchbhai et al. (1988) in buffalo calves (1.004 cm), Banubakode (1992) in cattle (1.22±0.008 cm) and Gelatt (2007) in cow (1.20 cm). These variations in the thickness of the lens may be due to the continuous addition of the new lens fibers by the process of transformation of the epithelial cells in to the lens fibers (Prince et al., 1960) or may be due to species variation or age of the animals.

4.8.1.3 Diameter

The overall mean value of the diameter of the lens of both the sides in adult surti buffalo was 1.845±0.005 cm. The mean value of diameter in the right lens was 1.84±0.02 cm with the range between 1.68 to 2.00 cm and, it was 1.85±0.02 in the left lens cm with the range between 1.69 to 2.00 cm.

The present observations are in accordance with the observations of Banubakode (1992) in cattle (1.84±0.009 cm) and Gelatt (2007) in cow (1.87 cm). Prince et al. (1960) in exotic cattle (1.95 cm) mentioned the higher mean value of the lens diameter than that of the present study. However, Martin and Anderson (1981) in
exotic cattle (1.72 cm) and Panchbhai et al. (1988) in buffalo calves (1.47 to 1.84 cm) reported the lower mean value than the present study. Cruickshank et al. (1968) described that the size of the lens increases gradually due to the formation and addition of the new lens fibers from the epithelial cells. This may be the reason for the variation observed in the diameter of the lens.

4.8.2 Histology

In the present study of adult surti buffalo, the lens was observed to be composed of three components viz. (1) the capsule, (2) the epithelium and (3) the lens fibers.


4.8.2.1 Capsule

The capsule was the outermost covering of the lens and it was homogenous in appearance and PAS positive membrane (Fig. 21). These observations are in concurrence with those of Foster (1962) in human beings, Martin and Anderson (1981) in cattle Banubakode (1992) in cattle, Dellmann (1993) in cattle and Gelatt (2007) in domestic animals.

The mean thickness of the anterior capsule and that of the posterior capsule were 47.28 µm and 9.9 µm respectively in the present study. These observations are more or less similar to the observations of Prince et al. (1960) in domestic animals where the anterior capsule was 40.00 µm and posterior capsule was 6.00 to 12.00 µm thick.
4.8.2.2 Epithelium

During the present work, it was observed that there was a single layer of epithelial cells below the capsule in the anterior portion of the lens (Fig. 23). These cells were cuboidal near the anterior pole but towards the equator, they became elongated to form columnar cells and transformed into the lens fibers (Fig. 22). These observations are similar to those of Prince et al. (1960) in cattle, Martin and Anderson (1981) in exotic cattle, Banubakode (1992) in cattle, Dellmann (1993) in cattle and Gelatt (2007) in domestic animals.

4.8.2.3 Lens fibers

The present study showed that the lens fibers constituted the main body of the lens and the newly formed lens fibers were observed to be nucleated while the older lens fibers were without nucleus (Fig. 22). These observations are in accordance with the observations of Banubakode (1992) in cattle, Dellmann (1993) in cattle and Gelatt (2007) in domestic animals.

Regarding the general histological structures of lens, there is no variation between the adult surti buffalo and the other different species.

4.9 STATISTICAL ANALYSIS

After application of student’s paired ‘t’ test, the following observations were obtained.

The echobiometrical mean values of anterior chamber depth, antero-posterior depth of the lens, depth of vitreous chamber and antero-posterior axis of the globe in the right eye balls did not show significant difference from the left eye balls at 5% level of significance (Table no. 1).
The biometrical mean values of the weight, antero-posterior axis, horizontal axis, and vertical axis of the right eye balls did not show significant difference with the left eye balls at 5% level of significance (Table no. 2).

The biometrical mean values of the vertical diameter and horizontal diameter of the cornea were not significantly different in right and left eye balls at 5% level. The thickness of cornea at the center and at the periphery also showed non significant difference (Table no. 2).

The weight, maximum diameter and thickness of the lens showed non significant difference between the right and left eye balls at 5% level (Table no. 2).

The micrometrical mean values of the total thickness of cornea, thickness of epithelial layer, thickness of stroma, thickness of descemet’s membrane and thickness of endothelium did not show significant difference between the periphery and center of the cornea in each eyeball at 5% level (Table no. 3).

The micrometrical mean values of choroid at the center showed significant difference to that values of choroid at the periphery at 5% level of significance. However, the micrometrical mean values of center and peripheral retina did not show significant difference. Similarly the number of epithelial cell layers showed non significant difference between the periphery and center of the cornea in eye balls (Table no. 3).
CHAPTER - 5
SUMMARY AND CONCLUSIONS

The present study entitled “Gross and Histomorphological Study on Eye Ball of the adult Surti Buffalo (Bubalus bubalis)” was carried out at the Department of Veterinary Anatomy and Histology in collaboration with the Department of Veterinary Surgery & Radiology and Department of Veterinary Pathology, C.V.Sc.&A.H., A.A.U., Anand, Gujarat. For this study, 20 eye specimens (10 right and 10 left) of buffaloes were used for the sonoanatomy, gross and histomorphological evaluations. The echobiometrical, biometrical and micrometrical measurements of the different parameters of the eyeball including cornea, sclera, choroid, retina and lens were recorded with the help of the scientific weighing balance, Vernier callipers, graduated eye piece and ultrasound machine. The histological sections (5 to 7 µm thickness) were stained with Haematoxylin and Eosin Stain for routine staining and Masson’s trichrome stain and Periodic Acid Schiff’s stain for special staining.

The following conclusions were drawn from the present study of gross and histomorphology on the eye ball of adult surti buffalo:

1. The ultrasonography of the eye balls showed that the eye balls were appeared as ovoid structures with anechoic contents such as aqueous humour, vitreous body and lens. The cornea, anterior and posterior lens capsule, iris, ciliary body and corpora nigra were appeared as echogenic substances.

2. The echobiometrical mean values of the anterior chamber depth, the antero-posterior depth of the lens, the vitreous chamber depth and the antero-posterior depth
of the right & left eye balls were 0.33±0.02 & 0.32±0.01 cm, 1.04±0.02 & 1.05±0.02 cm, 1.63±0.05 & 1.64±0.05 cm and 3.13±0.05 & 3.14±0.05 cm respectively.

3. The biometrical mean values of the weight, the antero-posterior axis, the horizontal axis and the vertical axis of the eye balls in the right & left eye balls were 31.15±0.85 & 31.17±0.76 gm, 3.67±0.04 & 3.67±0.04 cm, 4.04±0.03 & 4.04±0.03 cm and 4.02±0.04 & 4.00±0.02 cm respectively.

4. Histologically, the sclera can be subdivided into three layers such as (i) episclera (ii) sclera proper and (iii) lamina fusca. The micrometrical mean values of the thickness of the sclera at the periphery was 445.96±23.05 µm and that of the center was 856.95±33.84 µm.

5. The cornea was an elliptical transparent membrane having a broader end at the medial side and a more pointed end at the lateral side. The histological structures of cornea was composed of four layers. These were (i) Anterior epithelial layer (ii) Corneal stroma (iii) Descemet’s membrane (iv) Endothelial layer. The Bowman’s membrane of cornea was not evident in the present study. The biometrical and micrometrical mean values of the thickness of cornea were non significantly higher at the periphery than that of the center.

6. The biometrical mean values of the vertical diameter and the horizontal diameter of cornea in the right & left eye balls were 2.37±0.03 & 2.37±0.02 cm and 2.89±0.04 & 2.92±0.04 cm respectively. The biometrical mean value of the thickness of cornea was 1.11±0.08 mm at the center and was 1.29±1.29 mm at the periphery.

7. The micrometrical mean values of the thickness of the epithelial layer and number of the epithelial layers of the cornea at the periphery & at the center were 99.92±5.32 & 98.67±4.49 µm and 9.42±0.11 & 9.29±0.11 respectively. The mean values of the thickness of the corneal stroma, Descemet’s membrane and corneal
endothelium at the periphery & at the center were 667.32±67.27 & 657.59±63.05 µm, 18.79±2.36 & 18.62±2.31 µm and 5.08±0.37 & 4.95±0.35 µm respectively. The mean value of the total thickness of the cornea was 798.607±69.17 µm at the periphery and it was 789.13±64.20 µm at the center.

8. The choroid was composed of four layers such as (i) Suprachoroid (ii) Large vessel layer (iii) Tapetum (iv) Choriocapillary layer. The micrometrical mean thickness of the choroid was higher at the center than that of the peripheral section. The micrometrical mean thickness of the choroid was 76.55±3.72 µm at the center of the choroid whereas, in the peripheral section of choroid, it was 48.86±1.78 µm.

9. The irido-corneal angle was the area located at the periphery of the anterior chamber and formed by the junction of the corneoscleral tunic (Limbic zone), base of the iris and anterior ciliary body. The irido-corneal angle of the eye was comprised of the pectinate ligament, the ciliary cleft, the trabecular meshwork (uveal and corneoscleral) and the angular aqueous plexus.

10. Retina was composed of ten layers such as (i) Pigmented epithelium (ii) Layer of rods and cones (iii) External limiting membrane (iv) Outer nuclear layer (v) Outer plexiform layer (vi) Inner nuclear layer (vii) Inner plexiform layer (viii) Ganglion cell layer (ix) Nerve fibers layer (x) Internal limiting membrane.

11. The mean thickness of the retina in the center of the tunic was non significantly higher than that in the periphery of the tunic. The individual thickness of retina near to the optic nerve may be very high due to increasing the thickness of nerve fibers. The mean thickness of retina in the center of the tunic was 177.56±10.72 µm and that of retina in the peripheral section of the tunic was 120.24±15.40 µm.

12. Retina usually detached from the retinal pigment epithelium or from choroid. It may be due to faulty processing of tissue during preparation of blocks and
sectioning. In more than 60-70 % of the samples, it was found that retina took a convex appearance vitreally which was looking like a fold.

13. The lens was a transparent, soft and biconvex substance with the convexity more in the posterior surface than the anterior surface. Histologically, the lens was composed of three components namely (i) capsule, (ii) simple cuboidal epithelium and (iii) fibers.

14. The mean values of the weight, the thickness and the diameter of the lens were $2.52\pm0.07$ gm, $1.32\pm0.01$ cm and $1.84\pm0.02$ cm respectively in the right eye balls. The mean value of the weight, the thickness and the diameter of lens were $2.53\pm0.07$ gm, $1.32\pm0.01$ cm and $1.85\pm0.02$ cm respectively in the left eye balls.
REFERENCES
REFERENCES


Table no. 1: Statistical analysis of echobiometrical observations of 20 eye balls in adult surti buffaloes

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SE: Standard error, C.V. %: Percentage of coefficient of variation, Superscript (ab): statistically non-significant at 5 % level
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SE : Standard error, C.V% : Percentage of coefficient of variation, (c) : center of cornea, (p) : periphery of cornea, superscript (ab) : statistically non-significant at 5 % level
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<td>Total thickness (µm)</td>
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<td>Total thickness (µm)</td>
<td>94.57 to 149.33</td>
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APPENDIX II
Fig. 1: Ultrasound machine (e-saote MY Lab five VET).

Fig. 2: Echobiometry of eye ball of surti buffalo with the help of an ultrasound linear transducer probe (Transcorneal technique).

Fig. 3: Biometrical measurement of eye ball of surti buffalo with the help of digital Vernier callipers.

Fig. 4: Biometrical measurement of lens of surti buffalo with the help of digital Vernier callipers.

Fig. 5: Micrometrical measurement of cornea of surti buffalo with the help of Graduated eye piece.

Fig. 6: Micrometrical measurement of tunics of eye ball of surti buffalo with the help of Graduated eye piece.
Fig. 7: Sonograph showing the different echobiometrical parameters of eye ball of surti buffalo.
(D1) Anterior chamber depth  
(D2) Antero-posterior depth of lens  
(D3) Vitreous chamber depth  
(D4) Antero-posterior depth of eye ball.

Fig. 8: Sonograph showing the different components of eye ball of surti buffalo
(C) Cornea  
(AC) Anterior chamber  
(L) Lens  
(VC) Vitreous chamber  
(OD) Optic disc  
(*) Granula iridis  
(1) Anterior capsule  
(2) Posterior capsule  
(3) Iris  
(4) Ciliary body
Fig. 9: Eye ball of surti buffalo (front viewed) showing (1) Elliptical pupil, which is visible through transparent cornea (2) Limbus (3) Sclera (4) Iris, which is visible through transparent cornea and (*) Iris granules

Fig. 10: Eye ball of surti buffalo (side viewed) showing (1) Optic nerve at the posterior surface (2) Posterior ciliary artery (3) Cornea (4) Limbus and (5) Sclera

Fig. 11: Eye ball of surti buffalo showing the vortex veins (blue arrows) present at the surface, posterior to the equator of the eye ball.

Fig. 12: Half section of Eye ball showing (1) Cornea (2) Lens (3) Vitreous body
Fig. 13: Half section of the eye ball of surti buffalo (lens, aqueous humour and vitreous body were removed) showing (1) Cornea (2) Pupil (3) Iris (4) Limbus (5) Ciliary body (6) Junction between retina and ciliary body (7) Retina (8) Retinal blood vessel (9) Choroid and (10) Sclera.

Fig. 14: Lens of surti buffalo which is transparent and soft (before fixation).

Fig. 15: Lens of surti buffalo (after fixation) showing the biconvex surface, (A) anterior surface, (P) Posterior surface, (E) Equator and (ZF) Zonulary filaments.
Fig. 16: Photomicrograph of cornea of surti buffalo (150X magnification) stained with H&E stain showing the different layers such as (AE) Anterior epithelium, Corneal stroma, (DM) Descemet’s membrane and (PE) Posterior Endothelium.

Fig. 17: Photomicrograph of cornea of surti buffalo (300X magnification) stained with H&E stain showing Anterior epithelium, Corneal stroma, (SC) Superficial squamous cells, (BCC) Basal columnar cells, (BM) Basement membrane and (NF) Nuclei of fibroblasts.
Fig. 18: Photomicrograph of cornea of surti buffalo (300X magnification) stained with H&E stain showing (NPE) Nuclei of Posterior endothelium, (DM) Descemet’s membrane, (NF) Nuclei of fibroblast cell and some portion of Corneal stroma.

Fig. 19: Photomicrograph of cornea of surti buffalo (300X magnification) stained with Masson’s trichrome stain showing layers of anterior epithelium and green coloured stained collagenous bundles of stroma.
Fig. 20 (A) : Photomicrograph of sclera of surti buffalo (30X magnification) stained with Masson’s trichrome stain showing episclera, sclera proper, lamina fusca, choroid and (BV) Blood vessels.

Fig. 20 (B) : Photomicrograph of tunic of surti buffalo (30X magnification) stained with Periodic Acid Stain showing cornea, sclera, limbus, (FC) fornix of conjunctiva, iris, ciliary processes, (P) pectinate ligament and (TB) Trabecular meshwork
Fig. 21: Photomicrograph of lens of surti buffalo (75X magnification) stained with PAS stain showing (1) Capsule and (2) Fibers.

Fig. 22: Photomicrograph of lens of surti buffalo (300X magnification) stained with H&E stain showing (AE) Anterior epithelium, (GZ) Germinal zone, (NNF) Nuclei of new fibers and (NOF) Nuclei of old fibers.

Fig. 23: Photomicrograph of lens of surti buffalo (300X magnification) stained with H&E stain showing Capsule and (AE) anterior epithelium of lens.
Fig. 24: Photomicrograph of ciliary body of surti buffalo (75X magnification) stained with H&E stain showing (CP) Ciliary processes, (BV) Blood vessels and (M) Melanocytes.

Fig. 25: Photomicrograph of ciliary processes of surti buffalo (750X magnification) stained with H&E stain showing (1) Non-pigmented epithelium (2) Pigmented epithelium
Fig. 26: Photomicrograph of iris of surti buffalo (30X magnification) stained with H&E stain showing (1) Iris (2) Tip of iris (3) Cornea and (4) Anterior chamber.

Fig. 27: Photomicrograph of iris of surti buffalo (75X magnification) stained with H&E stain showing Stroma, (PPE) Posterior pigmented epithelium, (APE) Anterior pigmented epithelium, (BV) Blood vessels and (S) Smooth muscle.
Fig. 28: Photomicrograph of the tunics of eye ball of surti buffalo stained with H&E stain showing Retina, Choroid and Sclera (150X magnification).

Fig. 29: Photomicrograph of Retina of surti buffalo (300X magnification) stained with H&E stain showing:
1. Internal limiting membrane,
2. Nerve fibers layer,
3. Ganglion cell layer,
4. Inner plexiform layer,
5. Inner nuclear layer,
6. Outer plexiform layer,
7. Outer nuclear layer,
8. Outer limiting membrane,
9. Layer of rods and cones,
10. Pigmented epithelium and
11. Choroid.
Fig. 30: Photomicrograph of tunics of eye ball of surti buffalo (300X magnification) stained with H&E stain showing Retina, Choroid, Sclera, (CC) Choriocapillary, (TL) Tapetum lucidum, (M) Melanocytes and (BV) Blood vessel.

Fig. 31: Photomicrograph of tunics of eye ball of surti buffalo (750X magnification) stained with H&E stain showing the convergence of the nerve fibers of retina at the optic disc, some portion of choroid and optic nerve.
**Fig. 32**: Photomicrograph of tunics of eye ball of surti buffalo (150X magnification) stained with H&E stain showing the layers of retina, choroid and some portion of sclera, (TL) Tapetum lucidum and (M) Melanocytes.

**Fig. 33**: Photomicrograph of tunics of eye ball of surti buffalo (300X magnification) stained with H&E stain showing (RPE) heavily pigmented epithelium where tapetum is absent, (CC) choriocapillary and (M) melanocytes.
Fig. 34: Photomicrograph of tunics of eye ball of surti buffalo (75X magnification) stained with H&E stain showing (1) Retina (2) Choroid (3) Sclera (4) Ciliary body and (*) Ora ciliaris retinae.

Fig. 35: Photomicrograph of tunics of eye ball of surti buffalo (75X magnification) stained with H&E stain showing (1) Retina, (2) Choroid, (3) Sclera and (4) Folding of retina.
Fig. 36: Photomicrograph of Optic nerve of surti buffalo (75X magnification) stained with H&E stain showing (1) Optic disc, (2) Lamina cribrosa, (3) Retina, (4) Choroid, (5) Sclera and (6) Optic nerve.

Fig. 37: Photomicrograph of Optic nerve of surti buffalo (75X magnification) stained with H&E stain showing (1) Bergmeister’s papilla, a remnant of hyoid artery, (2) Retinal blood vessel, (3) Optic disc and (4) Optic nerve.