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ORIGINAL PAPER

Histomorphometric analysis of the choroid of donkeys, buffalos, camels and dogs

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Abstract
Aim The present study was carried out to investigate the morphological and histomorphometric characters of choroid in donkeys, buffalos, camels and dogs.
Results The findings of the study revealed that, macroscopically, the choroid was consisted of two areas in all studied animals, except in camel which consists of one area. Histologically, the choroid consists of five layers. Interestingly, the anterior borders of all investigated animals were free of pigments except in camel. Morphometric analysis revealed significant species differences in the mean total thickness of the choroid and its different layers. In addition, significant differences were also found between the ratios of the means of different layers to the total thickness of the choroid.
Conclusion In conclusion, these variations might be related to the different lifestyles and visual behavior of the investigated animals.

Keywords Choroid · Vascular tunic · Domestic animals

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Introduction

The choroid is a dark, pigmented, vascular membrane located between the sclera and the retina. It lines the sclera from the optic nerve almost to the corneoscleral junction [1–3].

In domestic animals, the choroid histologically subdivided into five layers, beginning with the outermost layer; they are the suprachoroid layer (*Lamina suprachoroidea*), the vessel layer (*Lamina vasculosa*), the layer of *Tapetum lucidum*, forms the larger colored area of choroid wall, the capillary layer (*Lamina choriocapillaris*) and the elastic membrane (*Lamina basalis,* Bruch's membrane or glassy membrane) [4–12].

Some of the eye affections including retinal detachment, intraocular hemorrhage and high intraocular pressure may occur as a result of choroid diseases [13–15]. Although several approaches were conducting for studying the choroid morphology, structure, pigmentation and function, scarce information is available about the histomorphometric, scanning electron microscope and immunohistochemistry of choroid. Therefore, the present study was undertaken to investigate the detailed morphological, morphometric and protein expression pattern in choroid of donkeys, camels, buffalos and dogs.

Materials and methods

Collected samples

The present work was carried out on five eyeballs from each of the following animals: donkey (*Equus asinus*), buffalo (*Bus bubalis*), camel (*Camelus dromedarius*) and dog (*Canis canis*). All samples were obtained from adult and clinically healthy animals of both sexes. The eyeballs of buffalos and camels were collected from Assiut slaughter houses, while those of donkeys and dogs were obtained from the dissecting room of the Department of Anatomy and the extracted eyeballs were weighed in grams. The total volume of each eyeball was determined using fluid displacement. After eyeball dissection, the weight and volume of the vascular tunic and choroid were measured. The surface area of the vascular tunic as well as the choroid was measured using the statistical diagrammatic paper.

Electron microscopy

For scanning electron microscopy (SEM) procedures, we followed what has been previously published [16, 17]. The specimens were then examined and photographed using JSM-5400 LV Scanning Electron Microscope in the Electron Microscope Center of Assiut University. The choroid was scanned on its cut surface to visualize its layers.

Immunohistology

For paraffin tissue sections, samples were fixed in 2% PFA for 1 day, dehydrated in ascending concentrations of ethanol in PBS (25, 50, 70 and 100%) and transferred in xylene (100%) for 5 min before embedding in paraffin (60 °C) overnight. With a rotational microtome, sections of 5 µm were cut, incubated in ethanol (100, 70, 50 and 25%) and stained with Trichrome (Merck, Germany) [18].

For Epon–araldite embedding, small tissue blocks were taken from the choroid and fixed in paraformaldehyde–glutaraldehyde solution in phosphate buffer. Specimens were postfixed in 1% osmium tetroxide for 1 h, washed in 0.1 M phosphate buffer (7.3 pH), then dehydrated in graded ethanol and embedded in Epon–araldite mixture. Semithin sections were cut (1 µm thick) and stained with toluidine blue and examined microscopically.

For the immunohistochemical studies, we followed previously published protocol [19]. Anti-smooth muscle actin antibody (Sigma-Aldrich) was used.

Imaging

Sections were examined with light microscope and photographed. In addition, some morphometric measurements were made using an image analysis system (Leica Q500). These measurements include the thickness of the choroid as well as *Tunica vasculosa* and *Tapetum Lucidum*.

Statistics

All measurements were statistically analyzed using a one-way ANOVA. Descriptive statistics are given as mean ± SD (standard deviation).

Results

The choroid represents the posterior portion of the vascular tunic (caudal uvea). It represents variable relative volume in relation to the eyeball (Table 1). Among studied animals, the volume percentage of the choroid/total eyeball volume is the highest in dogs (about 2.4%), followed by camels (about 2.3%), donkeys (about 2.1%) and buffaloes (about 1.8%) (Fig. 1e, Table 2). The absolute and relative surface area of the choroid in relation to the total vascular tunic shows slight variation in the studied animals (Fig. 1f, Tables 1, 2). Its relative surface area is the highest in buffaloes (about 71.28%) followed by donkeys (about 67.13%), camels (about 66.09%) and dogs (about 64.24%).

The choroid is a darkly pigmented thin layer extending from the optic disk to the ciliary ring (Fig. 1d). The most firm areas of attachment between the choroid and the sclera are detected at the *Lamina cribrosa* (area of exit of optic nerve fibers) and at the entry of the blood vessels into the choroid. The *Lamina*

Table 1 Mean total measurements of the eyeball. vascular tunic and choroid

	Donkey	Buffalo	Camel	Dog
Absolute volume (cm^3) of eyeball	32.6 ± 3.27	34.2 ± 1.81	29.9 ± 1.96	5.4 ± 0.51
Absolute volume (cm^3) of vascular tunic	2.5 ± 0.38	2.3 ± 0.36	2.1 ± 0.32	0.5 ± 0.07
Absolute volume (cm^3) of choroid	0.7 ± 0.23	0.6 ± 0.13	0.7 ± 0.11	0.1 ± 0.04
Absolute surface area (cm^2) of vascular tunic	44.09 ± 3.5	42.07 ± 3.5	43.51 ± 3.7	11.44 ± 0.9
Absolute surface area (cm^2) of choroid	29.60 ± 2.1	29.99 ± 2.2	28.76 ± 2.3	7.35 ± 0.6
Absolute surface area (cm^2) of tapetum nigrum	16.59 ± 1.1	16.28 ± 1.2	28.76 ± 2.3	5.10 ± 0.3
Absolute surface area (cm^2) of tapetum lucidum	13.01 ± 0.9	13.71 ± 0.8	–	2.25 ± 0.2
Total choroid thickness (μm)	191.43 ± 0.7	299.43 ± 0.34	169.32 ± 3.84	49.81 ± 0.14
Vascular layer thickness (μm)	69.58 ± 5.0	181.98 ± 9.09	41.35 ± 1.58	24.41 ± 0.27
Tapetum lucidum thickness (μm)	92.24 ± 0.94	86.47 ± 35.32	34.93 ± 93	11.38 ± 0.47

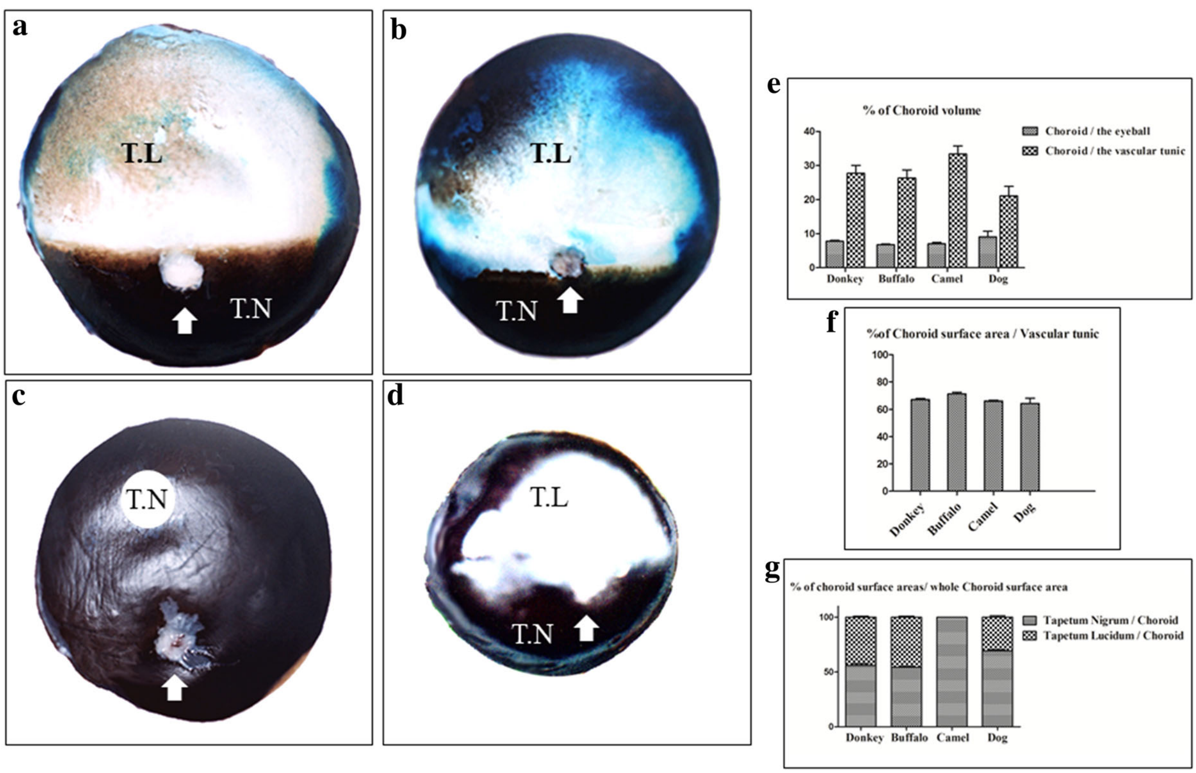

Fig. 1 The choroid of donkeys (**a**), buffaloes (**b**), camels (**c**) and dogs (**d**). The *Tapetum lucidum* (T.L.) is represented by a half circle in donkeys, while it is triangular with a dorsally directed apex in both buffaloes and dogs; however, it is absent in camels, and they have instead *Tapetum Nigrum* (T.N.). The optic papilla (arrow). Histograms to show relative choroid measurements in relation to the whole eyeball or vascular tunic (**e**–**g**). Compared to eyeball volume, the relative choroid volume of the dog is the largest among the studied animals, and the choroid of the buffalo is the smallest (**e**). The relative surface area of the choroid was the largest in buffalo and the smallest in dog (**f**). Relative surface area of both Tapetum Lucidum and Tapetum Nigrum in the studied animals showed that the largest Tapetum Lucidum found in buffalo, and the smallest found in dog (**g**). On the other hand the relative surface area of Tapetum Nigrum varies among the studied animals, and in camel it represents 100% of choroid (**g**)

Table 2 Relative measurements of the eyeball, vascular tunic and choroid

	Donkey	Buffalo	Camel	Dog
% Volume of vascular tunic/eyeball	7.63	6.57	6.88	8.33
% Volume of choroid/eyeball	2.17	1.80	2.30	2.40
% Volume of choroid/vascular tunic	27.71	26.31	33.36	21.08
% Surface area of choroid/vascular tunic	67.13	71.28	66.09	64.24
% Surface area of tapetum lucidum/vascular tunic	29.51	32.58	0	19.72
% Surface area of tapetum nigrum/vascular tunic	37.63	38.69	66.10	44.69
% Surface area of tapetum lucidum/choroid	43.98	45.66	0	30.69
% Thickness of tapetum/choroid	48.18	28.87	20.62	22.84
% Thickness of vascular layer/choroid	36.34	60.77	24.42	49

cribrosa is laterally located in donkeys and dogs, but it is central in position in buffaloes and camels Fig. 1a–d arrow).

As shown in Fig. 1a–c, in donkeys, buffaloes and dogs, the choroid consists of two areas: black area, Tapetum nigrum (T.N.), and a luster area, Tapetum lucidum (T.L.). On the other hand, the camel choroid is wholly black in color and lack of T.L. (Fig. 1c).

T.N. forms the majority of the choroid. T.L. is located in the upper part of the choroid just above the optic disk. It is represented by a half circle in donkeys (Fig. 1a), and it represents about 43.96% of the total choroidal area (Fig. 1g, Table 2). T.L. is triangular with a dorsally directed apex in both buffaloes (about 45.72% of choroidal area) and dogs (about 30.62% of choroidal area) (Fig. 1b, d). We noticed that the horizontal baseline of the tapetal area just touches the optic disk in donkeys [Fig. 1a arrow) and encloses it completely in buffaloes (Fig. 1b arrow)] and partially in dogs (Fig. 1d arrow).

To microscopically verify the choroid differences in studied animals, we sectioned the choroid and stained it with different histological stains. We measured the choroid thickness, and we found that the choroid varies in thickness in different studied animals. As shown in Table 1, choroid is the thickest in buffaloes (about 299.43 μm), followed by donkeys (about 191.43 μm) and camels (about 169.32 μm). In dogs, it is very thin (about 49.81 μm).

Structurally, the choroid consists of five layers from outside inward: suprachoroid (S.c.), vascular, choriocapillary and tapetal layers in addition to the Bruch's membrane.

The suprachoroid layer is represented by thin few interlacing collagen lamellae with many melanocytes in between.

The vascular layer (V) consists of large to medium-sized blood vessels interposed in a fine connective tissue stroma. It constitutes about 36.34% of the total choroidal thickness in donkey, 60.77% in buffaloes, 24.42% in camels and 49.00% in dogs (Table 2, Fig. 2e). This vascular layer appears usually to be arranged in two rows of vessels in buffaloes (Figs. 2b, 3b). The stromal connective tissue shows scattered melanocytes, which are numerous in donkeys, buffaloes and camels (Figs. 2a–c, 3a–c).

The *Tapetum lucidum* (T) varies in structure in different examined animals. It is fibrous in donkeys, buffaloes and camels, but it is cellular in dogs (Fig. 2a–c). The *Tapetum lucidum fibrosum* is composed of parallel interconnected bundles of collagen fibers with many horizontally arranged capillaries in between (choriocapillary layer). It constitutes about 48.18% of the total choroidal thickness in donkeys, 28.87% in buffaloes and 20.62% in camels (Table 2 and Fig. 2e). The collagen bundles (arranged in 13–15 layers) are thick with few capillary spaces in between in donkeys (Fig. 2 a red arrow) and closely arranged (10–12 layers) in buffaloes (Fig. 2b). In camels, the *Tapetum fibrosum* is weak consisting of fine loosely arranged collagen fibers (arranged in 9–12 thin layers). The camel is unique among the studied animals in that the retinal epithelium (R.E.) is heavily pigmented in the region of the *Tapetum fibrosum* (Figs. 2c, 3c). The *Tapetum lucidum cellulosum* of dogs constitutes about 22.84% of the choroidal thickness (Table 2 and Fig. 2e). It consists of regularly arranged layers of fusiform cells (arranged in 18–20 layers) intermingled with numerous melanocytes and vertically arranged capillaries (Figs. 2c, 3c).

The Bruch's membrane (Lamina basalis) is the inner most layer. It appears in the form of homogenous

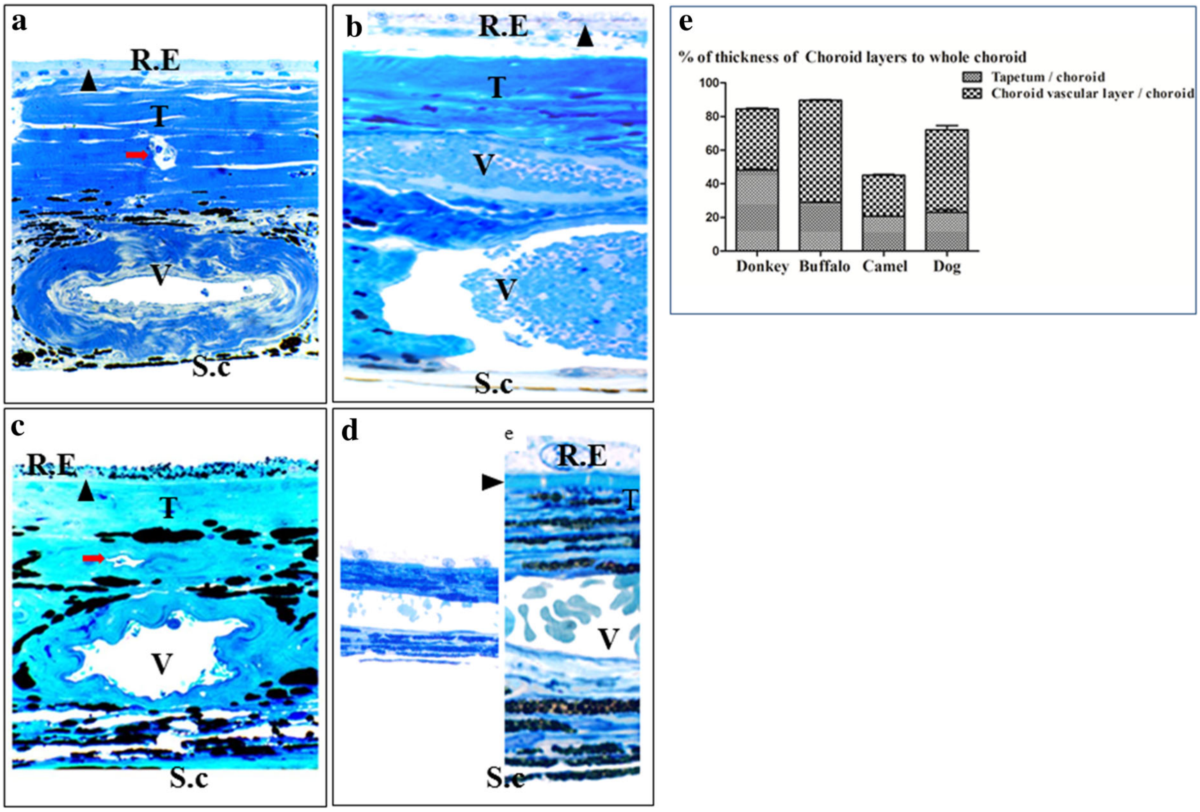

Fig. 2 Semithin sections in the tapetal area of the choroid of donkeys, buffaloes, camels and dogs, respectively, stained with toluidine blue. The choroid consists of five layers from outside inward: suprachoroid layer (Sc), vascular layer (V), tapetal layer (T), choriocapillary layer (arrows) and Bruch's membrane (arrowheads). The retinal epithelium (RE) is non-pigmented in all studied animals except camels. The *Tapetum lucidum* consists of bundles of collagen fibers in donkeys, buffaloes and camels (*Tapetum fibrosum*), and rows of fusiform-shaped cells (*Tapetum cellulosum*) in dogs. Note that the vascular layer in buffaloes is arranged in more than one row. (**f**) % of thickness of choroid layers to the whole choroid show that the thickset Tapetum is in donkey and the thinnest is in camel. The thickest choroid vascular layer is in buffalo, and the thinnest is in camel. (**a–d** ×400, **e** ×1000)

layer separating the retinal epithelium from the tapetal layer (Fig. 2a–c arrow head).

Detection of the smooth muscle actin in the smooth muscle cells revealed positive reaction in the vascular walls of all examined animals (Fig. 3a–c). In addition, free smooth muscle cells are occasionally demonstrated in the form of positive dark brown reaction in between the tapetal cells of dogs (Fig. 3e).

In cut sections, the central choroid appears to be consisting of four layers on examination by scanning electron microscope. These are from outside inward: suprachoroid, vascular, choriocapillary and tapetal layers. The Bruch's membrane is difficult to be demonstrated (Fig. 4a–d).

In all studied animals, the suprachoroid layer consists of few collagen lamellae that fasten the choroid to the lamina fusca of the sclera. The vascular layer is best developed in buffaloes (Fig. 4b) followed by dog (Fig. 4d) and donkeys (Fig. 4a). It consists of vascular network of large- and medium-sized blood vessels. In camels this layer is moderately developed and is represented by a relatively small-sized vascular network (Fig. 4c).

The tapetal layer is composed of mostly uniform collagen bundles which vary in thickness and number of layers in which they are arranged. In donkeys the tapetum is composed of thick, interlacing and loosely arranged collagen bundles with many intervening capillary network (Fig. 4a). In buffaloes, this layer is composed of parallel closely arranged collagen bundles with many capillaries in between (Fig. 4b). In camels, the tapetal layer is weakly developed and is

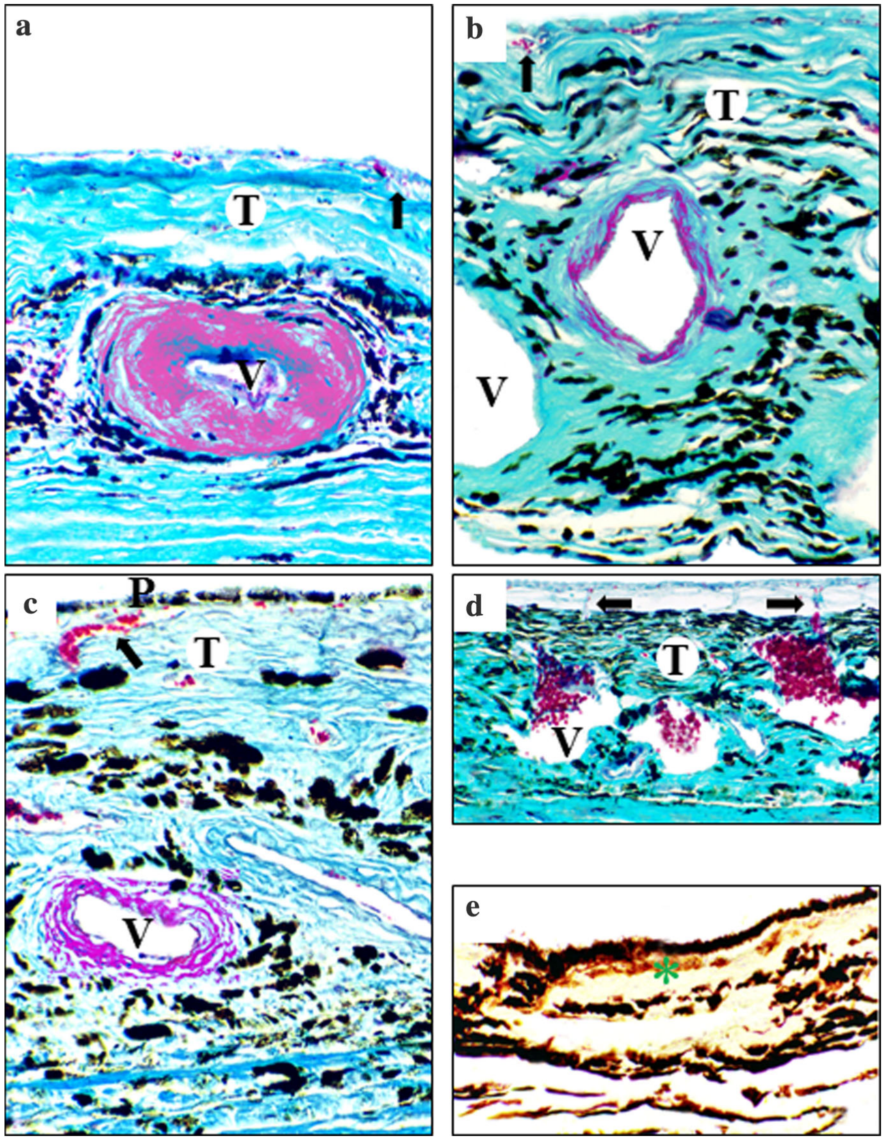

Fig. 3 Paraffin sections in the tapetal area of the choroid of donkeys, buffaloes, camels and dogs, respectively, stained with Crossmon's trichrome. The choroid consists of the known five layers, vascular layer (V), tapetal layer (T), choriocapillary layer (arrows). The *Tapetum lucidum* of camels consists of clearly demonstrated thin collagen bundles, but it is covered by pigmented retinal epithelium (P). The vessels of the choriocapillary layer are horizontal in all studied animals (**a**–**c**) except dogs (**d**) where they are vertically oriented. Immunohistochemistry showed a marked smooth muscle actin protein expression in the choroid of the dogs (**e**, arrow)

represented by very thin layer of fine collagen bundles with few intervening capillaries (Fig. 4c). In dogs, the tapetum appears in the form of well-packed layer, which is regionally fenestrated with some capillaries (Fig. 4d).

Discussion

In general, the current study reveals many anatomical and structural modifications in the vascular tunic of the eyeball in the studied animals. These modifications fit with the mode of life, feeding habits and the surrounding environments of these animals.

The absolute volume of the eye is variable among the studied animals. Surprisingly, the camels have the smallest eye among the large animals under study. In this connection, it has been mentioned previously that the size of the eye and those of its different structures influence the amount of light gathered [20]. It can be postulated that the size of the eye of an animal is inversely related to the amount of light in its surrounding environment.

The vascular tunic consists of choroid, ciliary body and iris. The choroid represents the posterior portion

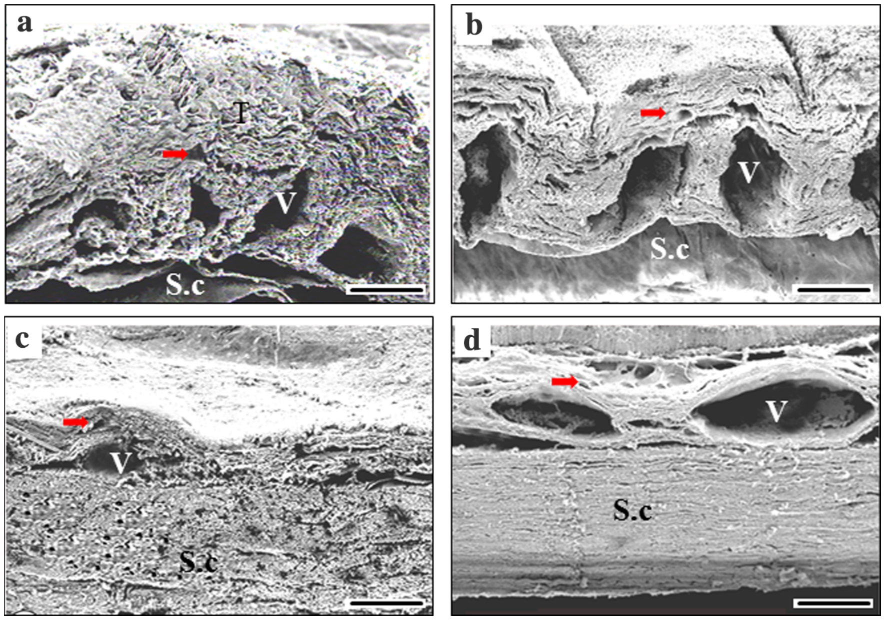

Fig. 4 Scanning electron micrograph of a cut surface of the choroid in donkeys (**a**), buffaloes (**b**), camels (**c**) and dogs (**d**). The tapetal layer (T) and vessel layer (V) vary in thickness and picture in different animals. Note the choriocapillary layer (arrows), suprachoroid layer (Sc) and sclera (S)

of the vascular tunic. With the exception of the camels, the choroid includes grossly two areas: black area (*Tapetum nigrum*) and luster area (*Tapetum lucidum*). The *Tapetum lucidum* provides the retinal photoreceptors with a second opportunity for stimulation by reflecting back the light that was not absorbed by them [21–24]. This tapetum is located in the dorsal fundus of the eye, which corresponds to the lower or foreground visual field area below the horizon as opposed to the sky area. Furthermore, the brightness of the sky above the horizon will be lighter than that of the ground, and the tapetum could reduce this difference at the retina so making it easier for the eye to operate [23].

The above statements may indicate that the position of the tapetum in an animal is closely related to the visual horizon in that animal. The visual horizon should be logically related to the posture of the head of the animal. In the present findings, the horizontal baseline of the tapetal area just touches the optic disk in donkeys, encloses it partially in dogs and completely in buffaloes. This means that the *Tapetum lucidum* is situated more dorsally in donkeys than in dogs and buffaloes, respectively. Taking the posture of the head in these animals in consideration, its long axis is nearly vertical on the ground surface in donkeys in comparison to its nearly horizontal axis in buffaloes and dogs. One can concludes that the visual horizon is less oblique in donkeys than in buffaloes and dogs. Accordingly, the tapetal area should be more dorsal in the former animal than the latter ones.

There is unanimous agreement concerning the structure of the mammalian choroid. The controversy lies only in the number of layers in which these structures are ordered. In agreement with many researchers [4, 11, 25], the choroid of the studied animals consists of five layers arranged from outside inward; suprachoroid, vessel layer, choriocapillary layer, tapetum and Bruch's membrane. Other authors have considered both vessel and choriocapillary layers in the choroid of ox as one unit called lamina propria [26]. On the other hand, others showed that the choroid consists of suprachoroid, stroma with large vessels, stroma with medium-sized vessels and tapetum and lastly the choriocapillary layer, excluding the Bruch's membrane from the layers of the choroid [15].

The *Tapetum lucidum* is either fibrous as seen in donkeys and buffaloes or cellular as the case in dogs. Both fibrous and cellular tapeta are in the form of well-ordered layers of collagen bundles and polygonal tapetal cells, respectively. The latter structures are of almost constant size and separated by almost constant spacing. In this respect, Denton 1970, 1971 and Land 1972 reported that if the higher refractive index material is of uniform size, orderly arranged and separated by specific and precise spacing, the small number of reflectance will sum in a more constructive manner and a higher overall reflectance will be achieved [27–29].

Ollivier et al. [20] have found that the extracellular structures in the *Tapetum fibrosum* and the endocellular ones in the *Tapetum cellulosum* are identical in their spatial arrangement. It therefore seems that tapetal types have the same general reflective mechanism regardless of their composition. The diverse chemical nature of the different tapeta may be responsible for determining different refractive indices and plays a role in the utilization of different wavelengths of light by animals according to their feeding behavior.

The number of layers in the *Tapetum lucidum* (fibrosum or cellulosum) varies from a studied animal to another. The tapetum of dogs demonstrates the presence of more layers than those of donkeys and buffaloes. In this concern, Denton [27] has indicated that in an ideal theoretical model of constructive interference, five layers of reflective material would give close to 75% reflection. Land [29] has stated that for most biologic systems, the reflectance will approach 100% after 10–20 layers. The difference between actual and ideal reflectance could be attributed to the interference of the existing blood vessels, nuclei of cells and imperfections in spacing and thickness of the reflective material [30, 31].

In the present study, the blood vessels, which penetrate into the tapetum to supply the choriocapillary layer, are vertically oriented in dogs in comparison to its horizontal arrangement in other studied animals. The vertical arrangement of these vessels in dogs has been reported to interfere as little as possible with its reflective function [32]. Moreover, Braekevelt has mentioned that the *Tapetum fibrosum* seems to be less rigid than the *Tapetum cellulosum* and thus does not force the choriocapillaris out of the reflective layer [33]. All these structural differences may indicate that the *Tapetum cellulosum* of dogs has a more reflective efficiency than the *Tapetum fibrosum* of donkeys and buffaloes and this may benefit the nocturnal nature of the dogs.

Previous studies have ascertained the absence of *Tapetum lucidum* in the camels [3, 34]. Although the choroid of the camels, in the present work, shows no *Tapetum lucidum* grossly, microscopically its central part demonstrates the presence of many layers of thin collagen bundles. However, the retinal epithelium is highly pigmented over this area. It can be postulated that the camels have a weakly developed, functionless *Tapetum lucidum fibrosum*, which is blocked by the covering pigmented retinal epithelium. This could be attributed to the bright conditions in which the camels normally live, where adequate light is available.

Our results demonstrate the presence of few occasional smooth muscle cells in the choroid of the dogs. The presence of extravascular smooth muscle cells in the choroid is a matter of controversy. While some authors have described free smooth muscle cells in the choroid of man and other species [23, 35], on the other hand some others deny their existence [36]. The choroidal smooth muscle cells in dogs may counteract the contraction of the ciliary muscle in this animal.

In conclusion, our study showed a marked difference of choroid in the studied animals. These variations might be related to the different lifestyles and visual behavior of the investigated animals.

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