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Ultrasonographic Characterization of Ocular Structures in Mules (*Equus mulus*)

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Abstract

Healthy eyes with good vision were important to any animal, to safely exist in their environments, for quality of life, and to successfully compete for food. The aim of present study was to set normal values for biometric intraocular dimensions in healthy mules for the right and left eyes in males and females. The study was conducted on clinically healthy mules (n=40) which were classified into two groups; male (n=20) and female mules (n=20). Complete clinical examination and ultrasonographic characterization of ocular structures were carried out for all clinically healthy mules. Biometric intraocular dimensions of the right eye and the left eye included anterior chamber depth (ACD), axial globe length (AGL), central lens thickness (CLT), lens pole diameter (LPD) and vitreous chamber depth (VCD), were described using ocular ultrasonography. It also showed normal intraocular structures of the right eye and the left eye that included, anterior chamber (AC), cornea (C), iris (I), lens (L), optic disk (OD), optic nerve (ON), retinochoroid unit (RCU), retrobulbar fat (RF), retrobulbar muscles (RM) and vitreous body (VB). Ocular ultrasonography reported no significant variations for biometric intraocular dimensions of the eye between male and female mules as well as between right and left eyes either in male mules or in female mules. The study reported normal values for ultrasonographic biometric intraocular dimensions of the right and left eyes in clinically healthy male and female mules.

KEYWORDS
biometric intraocular dimensions, clinical findings, intraocular structures, mules, ocular ultrasonography.

INTRODUCTION

The mule (*Equus mulus*) is a hybrid domestic animal that resulted from the breeding of a male donkey (Equus asinus) to a female horse (Equus caballus) (Neves *et al*., 2002). Mules were still widely used as a beast of burden or working equids for draft purposes in developing countries because they were considered to be strong and hardy animals, in some countries such as the U.S. there had been a growing interest in using mules also for recreational riding, racing and show purposes. The increase in mule use had created the need for more information on how to properly treat and care for them (Bazzano *et al*., 2020).

The eye is an elaborate organ whose primary function is to collect and focus light upon the photosensitive retina. It laid within a cone shaped cavity of the skull, the orbit, which housed the eyeball (globe) and other soft tissue structures. (Fails and Magee, 2018).

Healthy eyes and good vision were important to any animal, for quality of life, to safely exist in their environments, and to successfully compete for food. The ocular diseases produced considerable discomfort leading to poor weight gain, behavioral problems, poor performance and significant economic losses (Irby, 2017).

Ocular ultrasonography was reported to be a non-invasive, easy to perform, rapid, and economic diagnostic tool that allowed visualization of intraocular and retrobulbar structures (Abedellaah *et al*., 2018).

Ocular ultrasonography was of great advantage where direct opthalmoscopic examination was limited by the opacification within the cornea, aqueous humor, lens and vitreous humor such in case of corneal edema, ulcer, cataract or presence of intraocular mass. Ultrasonography allowed visualization and examination of the eye or its internal structure that would otherwise be difficult to assess due to eyelid swelling or prominence of the third eyelid (Hussein *et al*., 2021; Wafy *et al*., 2021).

The ultrasonographic appearance and dimension of the eye of horses (Grinninger *et al*., 2010; Mouney *et al*., 2012; Herbig and Eule, 2015) and of donkeys (Laus *et al*., 2013; Laus *et al*., 2014; Salavati *et al*., 2017; Hussein *et al*., 2021; Wafy *et al*., 2021) had been described. According to the authors knowledge, the ultrasonographic appearance and biometry of the mules' eyes had not been fully described. Therefore, the current study aimed to describe the ultrasonographic appearance of the normal mules' eyes as well as establishing the references values for biometric intraocular dimensions for the right and left eyes in males and females healthy mules.

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MATERIALS AND METHODS

Ethical guidelines

All experimental protocols were approved by Institutional Animal Care and Use Committee guidelines of Assiut University and Aswan University, Egypt that was in agreement with the Guide for Laboratory Animals Use and Care of the National Institutes of Health in USA (NIH publication No. 86-23, revised 1996).

Animals

The experiment was conducted on clinically healthy forty adult native Egyptian mules (Equus mulus) used for working purpose and belonged to private owners in Assiut governorate, Egypt. They belonged to both genders i.e. twenty males and twenty female mules. The animals age ranged between 5 to 8 years and their weight ranged between 260 and 350 kg. The mules were housed during the experiment under hygienic and sanitary protocol in clean stables and fed a balanced diet of mixed grain with hay and free access to water. No abnormalities were noted on the examined mules during the study.

Clinical examination

Clinical examination included mainly body temperature, pulse rate, respiratory rate, lymph nodes, mucous membranes, signs of dehydration and capillary refill time (CRT) were examined in all mules according to Garba *et al*. (2015).

Eye Ultrasonography

Ultrasonography was performed using an ultrasound machine (MyLabOne VET, Esaote, Italy) with a 5 to 11 MHz linear probe according to Hussein *et al*. (2021) and Wafy *et al*. (2021). The ultrasonographic examination was conducted on the left and right eyes in horizontal and vertical planes whereas the transducer was applied perpendicularly to the corneoscleral limbus by trans- palpebral technique after applying sufficient lubricant and under minimal pressure on the eye. The probe was applied on the eyelids with no or minimal pressure to get images of the ocular globe of both right and left eye. Several ocular parameters were evaluated by the ultrasound software including: anterior chamber depth (ACD) which measured from the cornea to the anterior surface of the lens capsule, central lens thickness (CLT), vitreous chamber depth (VCD), from the posterior surface of the lens capsule to the beginning of the retrobulbar region, axial globe length (AGL) or the meridian diameter, anterior-posterior axis from the cornea to the beginning of the retrobulbar region and lens pole diameter (LPD). After completion of the procedure the eyes were rinsed off with normal saline solution to remove coupling gel. All these measurements were taken by the same investigator.

Statistical analysis

All statistical analyses were performed using computer software (SPSS version 17.0, Chicago, USA). The data obtained were analyzed by independent-sample or paired t-test. The significance of differences between mean values at male and female mules or between the means at right eye dimensions and left eye dimensions was evaluated by Dunnett's test at $P<0.05$.

RESULTS

Clinical findings

All investigated mules of both groups exhibited normal body temperature, appetite and lymph nodes with absence of ataxia, diarrhea, cough and abnormal lung sounds. Signs of dehydration and mucous membranes paleness were not observed. The investigated mules had normal CRT (Table 1). The body temperature, pulse rate and respiration showed no significant changes ($P>0.05$) between male mules and female mules as shown in Table 1.

Table 1. Mean values (M±SD) of temperature, pulse and respiration in male mule group (n=20) and female mule group (n=20).

Groups	Temperature	Pulse (Beat/min)	Respiration (/min)
Male mule	37.36±0.31	31.03±2.66	12.63±2.08
Female mule	37.62±0.24	33.11±3.43	14.26±3.11

Significant when the values at male group compared with those of female mule group ($p<0.05$).

Ocular ultrasonographic findings

The technique of ocular ultrasonographic examination was feasible and all mules were tolerant to ultrasound examination of the eye without sedation or general anesthesia. Although the obtained images of the horizontal and vertical planes were similar, acquisition of the interpretable sonograms was more easily done in horizontal planes.

The ocular sonograms of all animals showed that the hyper echoic region represented the cornea, anterior lens capsule, posterior lens capsule and retino-choroid sclera complex. The cornea was represented as a thin hyperechogenic curved line that can be slightly flattened by exerting gentle pressure over the transducer. The anterior lens capsule was imaged as an echogenic convex line, the posterior lens capsule was visualized as as an echogenic concave line, and the lens in between was imaged as anechoic part. The posterior pole of the eye which included retina, choroid and the sclera could not be differentiated ultrasonographically and was identified as a hyper-echogenic convex structure. The iris appeared as moderately echogenic structures located abaxially to the lens.

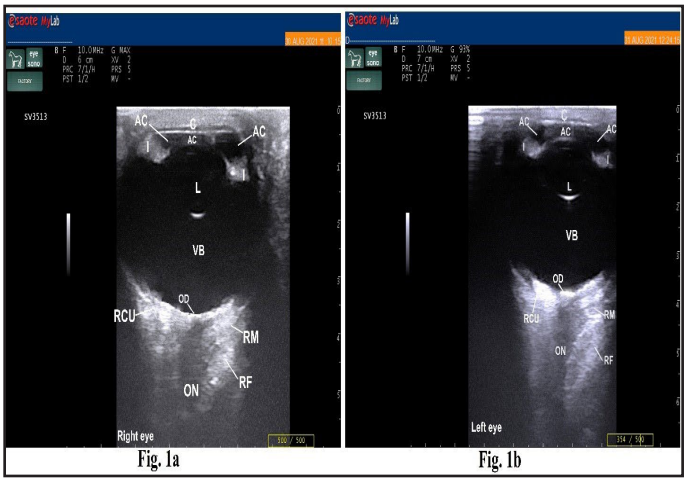

Fig. 1a Fig. 1b

Fig. 1. Transpalpebral B-mode ultrasonographic scan of a 6 years-old mule obtained in horizontal plane using 10 MHz linear array transducer showed normal intraocular structures of the right eye (Fig.1a) and the left eye (Fig. 1b). AC, anterior chamber; C, cornea; I, iris; L, lens; OD, optic disk; ON, optic nerve; RCU, retinochoroid unit; RF, retrobulbar fat; RM, retrobulbar muscles; VB, vitreous body.

The lens cortices and center (nucleus) as well as the aqueous and vitreous humors, were visualized as anechoic. The optical disc was identified as more echogenic area at the posterior ocular wall (Fig.1).

Biometric measurements of mules intraocular dimensions were reported in Tables 2-6 whereas they described dimensions of AGL, ACD, CLT, LPD and VCD. Biometric measurements of ocular dimensions of mules' eye revealed no significant changes either for AGL, ACD, CLT, LPD or VCD between eyes (i.e. right eye or left eye) of male mules and those of female mules (i.e. right eye or left eye). No significant changes were also reported between the right eye and the left eyes for these intraocular measurement either in male mules or female mules (Fig.2).

Table 2. Mean values (M±SD) of ultrasonographic parameters of the right eye and left eye structures in mixed mule group (n=40)

Groups	ACD (mm)	CLT (mm)	VCD (mm)	AGL (mm)	LPD (mm)
Right eye	3.97±0.37	11.1±0.71	19.83±1.46	36.97±1.59	17.58±0.78
Left eye	3.92±0.35	11.20±0.64	19.64±1.57	36.71±1.84	17.36±0.74

ACD: Anterior chamber depth. CLT: Central lens thickness. VCD: Vitreous chamber depth. AGL: Axial globe length. LPD: Lens pole diameter. *Significant when the values at right eye group compared with those of left eye (*p<0.05).

Table 3. Mean values (M±SD) of ultrasonographic parameters of the eye structures in male mule group (n=20)

Groups	ACD (mm)	CLT (mm)	VCD (mm)	AGL (mm)	LPD (mm)
Right eye	3.89±0.38	10.98±0.70	19.94±1.50	36.56±1.59	17.43±0.89
Left eye	3.95±0.27	11.02±0.50	19.57±1.51	37.22±1.88	17.04±0.71

ACD: Anterior chamber depth. CLT: Central lens thickness. VCD: Vitreous chamber depth. AGL: Axial globe length. LPD: Lens pole diameter. *Significant when the values at right eye compared with those of left eye group (*p<0.05).

Table 4. Mean values (M±SD) of ultrasonographic parameters of the eye structures in female mule group (n=20)

Groups	ACD (mm)	CLT (mm)	VCD (mm)	AGL (mm)	LPD (mm)
Right eye	4.04±0.37	11.22±0.73	19.72±1.49	37.38±1.55	17.72±0.66
Left eye	3.89±0.42	11.37±0.74	19.71±1.71	36.19±1.75	17.68±0.65

ACD: Anterior chamber depth. CLT: Central lens thickness. VCD: Vitreous chamber depth. AGL: Axial globe length. LPD: Lens pole diameter. *Significant when the values at right eye compared with those of left eye group (*p<0.05).

Table 5. Mean values (M±SD) of ultrasonographic parameters of the right eye structures in male mule (n=20) and female mule group (n=20)

Groups	ACD-R (mm)	CLT-R (mm)	VCD-R (mm)	AGL-R (mm)	LPD-R (mm)
male mule	3.89±0.38	10.98±0.70	19.94±1.50	36.56±1.59	17.43±0.89
female mule	4.04±0.37	11.22±0.73	19.72±1.49	37.38±1.55	17.72±0.66

ACD-R: Anterior chamber depth of the right eye. CLT-R: Central lens thickness of the right eye. VCD-R: Vitreous chamber depth of the right eye. AGL-R: Axial globe length of the right eye. LPD-R: Lens pole diameter of the right eye. *Significant when the values of right eye dimensions at male group compared with those of female mule group (*p<0.05).

Table 6. Mean values (M±SD) of ultrasonographic parameters of the left eye structures in male mule (n=20) and female mule group (n=20)

Groups	ACD-L (mm)	CLT-L (mm)	VCD-L (mm)	AGL-L (mm)	LPD-L (mm)
male mule	3.95±0.27	11.02±0.50	19.57±1.51	37.22±1.88	17.04±0.71
female mule	3.89±0.42	11.37±0.74	19.71±1.71	36.19±1.75	17.68±0.65

ACD-L: Anterior chamber depth of the left eye. CLT-L: Central lens thickness of the left eye. VCD-L: Vitreous chamber depth of the left eye. AGL-L: Axial globe length of the left eye. LPD-L: Lens pole diameter of the left eye. *Significant when the values of left eye dimensions at male group compared with those of female mule group (*p<0.05).

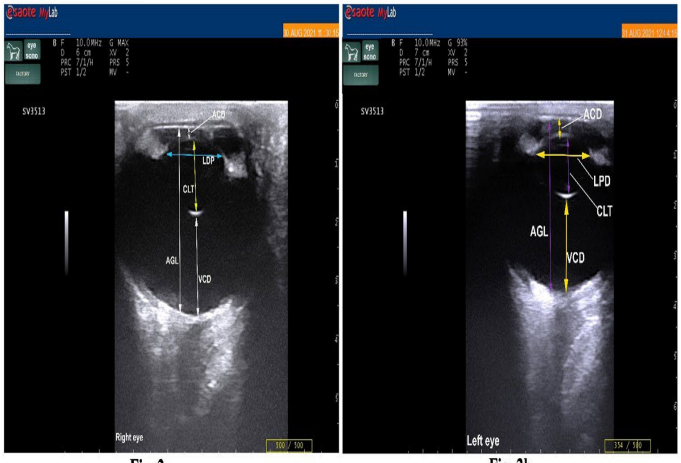

Fig. 2a Fig. 2b

Fig. 2. Transpalpebral B-mode ultrasonographic scan of a 6 years-old mule obtained in horizontal plane using 10 MHz linear array transducer. It showed biometric intraocular dimensions of the right eye (Fig.2a) and the left eye (Fig. 2b). ACD, anterior chamber depth; AGL, axial globe length; CLT, central lens thickness; LPD, lens pole diameter; VCD, vitreous chamber depth.

DISCUSSION

Given the stoic nature of donkeys and their hybrids, it was important to consider the significance of diagnostic testing modalities that could provide objective health status information beyond the basic physical examination findings. However, clinical pathology assays were also fraught with significant limitations because the results for donkeys, mules, and hinnies could be difficult to interpret, and transference of data from the horse is not always applicable (Goodrich and Behling-Kelly, 2019).

All investigated mules of both groups throughout the present work exhibited normal body temperature, appetite and lymph nodes with absence of ataxia, diarrhea, cough and abnormal lung sounds. Dehydration signs and mucous membranes paleness were not reported as well as clear improvement of CRT were reported in all animals. The body temperature, pulse rate and respiration showed no significant changes between male mules and female mules whereas they were within the references ranges reported by Gore *et al.* (2008); Garba *et al.* (2015).

Ocular ultrasonography is a safe, non-invasive and painless method of examining intraocular and retrobulbar structures that might be undetectable using ordinary light sources or ophthalmoscopy, especially whenever opacity of the transmitting media of the eye (cornea, aqueous humor, lens and vitreous humor) prevented a complete ophthalmic examination (Soroori *et al.*, 2009).

To the best of the authors' knowledge, there had been no study in veterinary medical literature about the ultrasonographic appearance and biometry of the normal mules' eyes, obtaining these measurements would serve as a base from which clinical examination of the mules' eyes might be done and could be a benchmark to diagnose some of the diseases and eye problems of mules. In the present study, all mules withstood eye ultrasound without sedation or general anesthesia. This was in agreement with the findings of Tripathi *et al.* (2018). Thus, sedation and general anesthesia, did not appear necessary in this circumstance. Therefore, anesthetic risks and additional costs were eliminated.

There are two techniques for ocular ultrasonography, transpalpebral through the eyelids and trasnscorneal which involves the transducer and coupling gel being applied directly to the corneal surface (Vali and Razeghi, 2019). In the current study, transpalpebral ultrasonography was performed to describe the normal intraocular morphology and to obtain normal echobiometric indices as previously done by Soroori *et al.* (2009); Hussein *et al.* (2021). However trans-corneal technique was used by Valentini *et al.* (2014); Tripathi *et al.* (2018). Although the trans-corneal technique produced high image quality; its main limitations included that the technique carried a risk of corneal damage, the acoustic

gel needed to be sterile, nonirritant and water soluble and this technique less well tolerated by the animal and usually necessitated the use of topical anesthetic drops and sedation (Valentini *et al*., 2010). The transpalpebral approach was well tolerated by the animals, and it allowed for the acquisition of well-defined non deformed images because minimal or no probe pressure was applied on the eyes and this technique may be desirable where the possibility exists of further damage to the cornea through direct probe contact (Hakim *et al*., 2021). The only disadvantage of transpalpebral scanning was the artifacts that might be generated by the eyelids (Hallowell and potter, 2010).

Generally, there was no difference was found in the ultrasonographic appearance of ocular structures between mules in the current study and between those in horses (Grinninger *et al*.,2010; Mouney *et al*., 2012; Herbig and Eule, 2015) and in donkeys (Laus *et al.* 2013; Laus *et al*., 2014; Salavati *et al*., 2017; Hussein *et al*., 2021; Wafy *et al*., 2021). The current study reported that the ocular sonograms of all mules either males or females showed that the hyper echoic region represented the cornea, anterior lens capsule, posterior lens capsule and retino-choroid sclera complex. The cornea was represented as a thin hyperechogenic curved line that can be slightly flattened by exerting gentle pressure over the transducer. The anterior lens capsule was imaged as an echogenic convex line, the posterior lens capsule was visualized as an echogenic concave line, and the lens in between was imaged as anechoic part. The posterior pole of the eye which included retina, choroid and the sclera could not be differentiated ultrasonographically and was identified as a hyper-echogenic convex structure. The iris appeared as moderately echogenic structures located abaxially to the lens. The lens cortices and center (nucleus) as well as the aqueous and vitreous humors, were visualized as anechoic. The optical disc was imaged as more echogenic area at the posterior ocular wall.

In the present study, biometric measurements of mules intraocular dimensions (AGL, ACD, CLT, LPD and VCD) were reported for the right and left eyes in both healthy male and female mules. These biometric measurements of ocular dimensions of mules' eye revealed no significant changes either for AGL, ACD, CLT, LPD or VCD between eyes (i.e. right eye or left eye) of male mules and those of female mules (i.e. right eye or left eye). No significant changes were also reported between the right eye and the left eyes for these intraocular measurement either in male mules or female mules. Similar findings were stated in bovine and equine (Tripathi *et al*., 2018), horses (Soroori *et al*., 2009), and donkeys (Wafy *et al*., 2021). Moreover, several studies had been conducted on the eye ultrasonography in different breeds of horses and different values had previously been reported for equine globe dimensions. The results varied between 3.0 and 6.8 mm for ACD, between 8.87 and 12.3 mm for CLT, between 17.37 and 24.48 mm for CLT, and between 32.9 and 43.1 mm for AGL (Herbig and Eule, 2015).

The biometric measurements of mules ocular dimensions in the present study tended to be smaller than those in studies involving full-sized horses (Grinninger *et al*., 2010; Mouney *et al*., 2012). On other side, similar values to the current study were reported in American miniature horse (Plummer *et al*., 2003), Caspian miniature horse (Soroori *et al*., 2009) and donkeys (Laus *et al*., 2013; Laus *et al*., 2014; Salavati *et al*., 2017; Wafy *et al*., 2021). The study provided the normal ultrasonographic guide (Echobiometry measurements) of the mules' eye by using a widely available and valuable diagnostic tool (ultrasonographic machine), which provided well standard line acquaintance for the study of pathologic conditions affecting the ocular components of mules. The study reported normal values for ultrasonographic biometric intraocular dimensions of the right eye and the left eyes either in clinically healthy male mules or female mules. Although many similarities for intraocular ultrasonographic findings were reported between horses, mules and donkeys, the use of biometric intraocular dimensions of the horse or those of donkeys to evaluate those of the mules might not be appropriate.

CONCLUSION

The study provides the normal ultrasonographic guides (Echobiometry measurements) of the mules' eye by using a widely available and valuable diagnostic tool (ultrasonographic machine), which provided well standard line acquaintance for the study of pathologic conditions affecting the ocular components of mules. The study reports normal values for ultrasonographic biometric intraocular dimensions of the right eye and the left eyes either in clinically healthy male and female mules.

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CONFLICT OF INTEREST

Authors declared that no conflict of interest exists.

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