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OPHTHALMOLOGICAL EVALUATION OF SHEEP FROM THE ALENTEJO REGION - PORTUGAL

(Avaliação oftalmológica de ovelhas da região do Alentejo - Portugal)

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ABSTRACT - Considering the importance of the flock of Alentejo sheep, the goal of this study was to determine the average values of eye examinations, evaluate the ophthalmoscopic features and fluorescein staining for Merino sheep from Alentejo region, in Portugal. The 48 eyes of 24 healthy, female sheep belonging to the sheep flock at the University of Évora were evaluated. The average values obtained for Schirmer tear test (STT) were 19.0 ± 5.22 mm/min, with a reduction of tear production varying according to the age of the animal, for tear pH were 7.7 ± 0.48 and for intraocular pressure (IOP) was 12.7 ± 3.03 mmHg. Fundus aspects were observed in the tapetal area with shades of blue, green and yellow. The optic disc was predominantly in the transition area between tapetal and non-tapetal regions of elliptical shape, with holoangiotic vascular pattern. None of the animals showed fluorescein staining on the ocular surface. In the context of the investigation, these data may be useful to improve the clinical approach to the species and reference standards of the exam's values.

Key words: IOP; merino sheep; ophthalmoscopy; STT; tear pH.

RESUMO - Considerando a importância do rebanho de ovinos do Alentejo, o objetivo deste estudo foi determinar os valores médios dos exames oftalmológicos, avaliar as características oftalmoscópicas e a coloração com fluoresceína para os ovinos Merino da região do Alentejo, em Portugal. Foram avaliados 48 olhos de 24 ovelhas saudáveis, do rebanho da Universidade de Évora. Os valores médios obtidos para o teste lacrimal de Schirmer (TLS) foram 19,0 ± 5,22 mm/min, com redução da produção lacrimal variando de acordo com a idade do animal, para o pH lacrimal foi de 7,7 ± 0,48 e para a pressão intraocular (PIO) foi de 12,7 ± 3,03 mmHg. No fundo de olho, observou-se a área tapetal com tons de azul, verde e amarelo. O disco óptico estava predominantemente na

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área de transição entre as regiões tapetal e não tapetal de formato elíptico, com padrão vascular holoangiótico. Nenhum dos animais apresentou coloração com fluoresceína na superfície ocular. No contexto da investigação, esses dados podem ser úteis para melhorar a abordagem clínica das espécies e padrões de referência dos valores dos exames.

Palavras-chave - oftalmoscopia; ovelha Merino; pH lacrimal; PIO; TLS.

INTRODUCTION

In Portugal, sheep fulfill an important economic role, especially in the Alentejo region, where they traditionally gather approximately half of the national flock, predominating the Merino breed their crosses (Ribeiro, 2012).

It is of great importance to study ophthalmic diseases in production animals, once they have an important economic impact (Ribeiro et al., 2010; Kulualp et al., 2018), as observed in the Infectious Keratoconjunctivitis disease in sheep (Razavizadeh and Razmyar, 2014; Akgül et al., 2017). Sheep, in particular, is still relevant as an experimental model in research (Gerometta et al., 2010). Thus, it is essential to obtain standard interval values for healthy sheep, regarding ophthalmologic test results, collaborating with an ophthalmological database (Ribeiro et al., 2010; Pigatto et al., 2011).

The semiological evaluation of the visual system of domestic animals requires examinations and tests with the aid of magnification, use of vital dyes, tear tests, and IOP measurement, in addition to other complementary exams (Gelatt et al., 2021). Given that the literature contains little reference to ocular parameters in sheep, studies and evaluations of normal values of ocular morphophysiology become relevant, mainly in regions where large flocks are concentrated, such as Alentejo, in Portugal. This study aimed to evaluate and determine the values that could be used as a reference for sheep of the Merino Alentejo breed in eye exams of IOP, STT, lacrimal pH, alongside the description of ophthalmoscopic and fluorescein staining features.

MATERIALS AND METHODS

This study was conducted at the Veterinary Hospital of the University of Évora, Portugal, following the criteria of the Association for Research in Vision and Ophthalmology (ARVO). The 48 eyes of 24 adult female Merino ewes belonging to the sheep flock at the University of Évora, were evaluated. The inclusion criteria for the animals that were chosen for this study consisted of being a healthy animal that was free of ophthalmic and systemic diseases, and also being fully vaccinated and dewormed. As

the animals studied were chosen at random, considering that the herd of the Veterinary Hospital of the University of Évora is composed almost entirely of female sheep.

Clinical evaluation and ophthalmological examinations (figure 1) were performed by the same clinician, during October 2017, between 9:00 and 12:00 a.m. The exams were performed in sheep stalls with their head restraint being performed smoothly, to avoid any movement at the time of the examination. The screening ophthalmological examination was performed with the aid of biomicroscopy, with a portable slit lamp (figure 1A) for the evaluation of the periocular region, ocular surface, anterior segment, and visual reflexes. If there were any abnormal findings after this procedure, the animal was excluded from the study. It was established to perform the examinations starting with the right eye and after this, progressing to the left eye of each animal. It was established to perform the examinations starting with the right eye and after this, progressing to the left eye of each animal. The variables STT, tear pH and IOP were measured, in this chronological sequence followed by the performance indirect ophthalmoscopy and sodium fluorescein strip test.

For STT, it was used commercial measuring strips, positioned on the lower eyelid in contact with the conjunctival fornix (figure 1B), maintained for a period of 60 seconds, then the strip was removed for numerical reading. The measurement of the tear pH was carried out by placing in the conjunctival sac (similar to STT) a tape of universal pH indicator paper (figure 1C), kept in contact with tears (60 seconds). The pH strip was read by comparing the colour of the tape, with a scale of colours provided by the manufacturer.

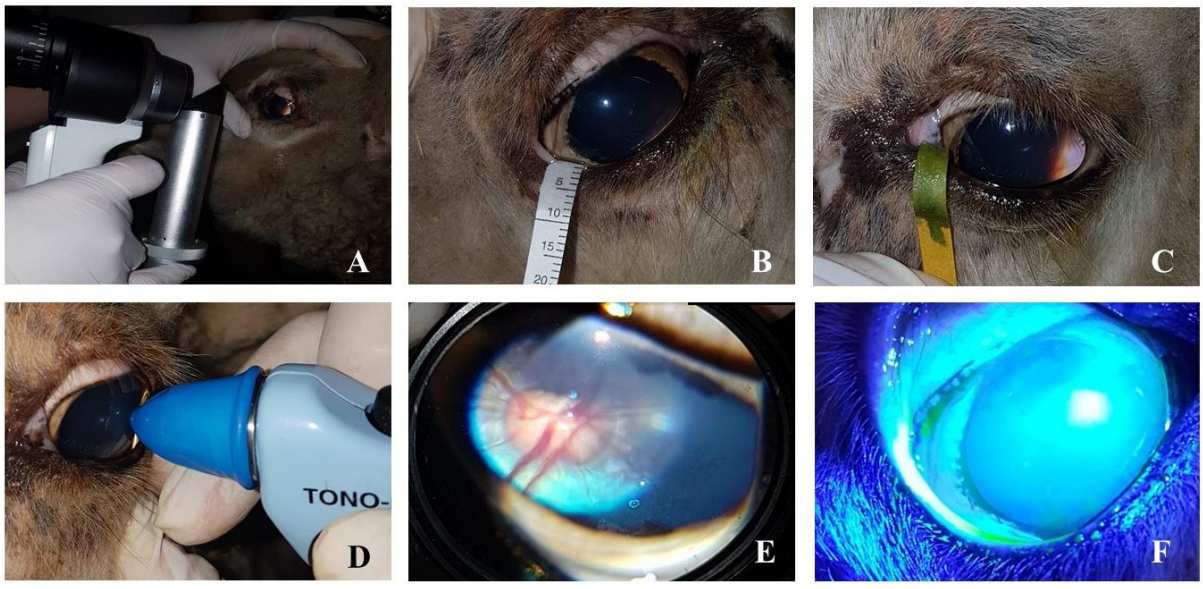

Figure 1 - Evaluation and eye exams. A (Slit lamp biomicroscopy), B (STT), C (tear pH), D (IOP), E (Fundoscopy) and F (Fluorescein test).

Prior to the IOP examination, a drop of anaesthetic of oxybuprocaine hydrochloride was instilled into the ocular surface of each eye. IOP measurement was performed using applanation tonometry, through subtle touches on the central region of the cornea (figure 1D). Only the values that reached a standard deviation of less than 5% on the device's display were considered for the purposes of statistical analysis. During the IOP measurement, the animals' heads were kept in a position that was higher than the height of the sternum, without offering any pressure in the neck region, to avoid errors in the interpretation of the results.

Two drops of 1% tropicamide were instilled on the ocular surface of both eyes to induce mydriasis, after the measurement of the IOP. After 30 minutes, the fundus of the eye was observed by indirect ophthalmoscopy, performed with the aid of a 20 D lens (figure 1E), and its characteristics were described. The integrity of the ocular surface in terms of the presence or absence of injury was observed by means of the fluorescein test and by using the cobalt blue filter from the slit lamp to enhance the possible lesions (figure 1F). The data that was obtained from the examinations and tests were processed and the descriptive analysis software RStudio was applied to the data.

RESULTS

The 48 eyes of 24 female sheep were evaluated. The mean age and weight of the sheep ± standard deviation was 6.0 ± 2.54 years and 52.0 ± 7.52 kg, respectively. In the screening ophthalmologic examination, none of the animals showed any abnormalities.

The mean values obtained for STT for all animals was 19.0 ± 5.22 mm/min. The mean tear pH was 7.7 ± 0.48 and for the IOP was 12.7 ± 3.03 mmHg. No significant differences were found between the left and right eyes. The mean, standard deviation, standard error, and coefficient of variation of the variables age, weight, STT, tear pH and IOP are shown in table 1.

Table 1 - Descriptive analysis of characteristics of weight, age and ophthalmological variables of Alentejo sheep.

Variables	A ± SD[1]	MSE[2]	VC[3]
Age (years)	6.0 ± 2.54	0.4	42.5%
Weight (kg)	52.0 ± 7.52	1.1	14.5%
Schirmer's Tear Test (mm/min)	19.0 ± 5.22	0.8	27.4%
Tear pH	7.7 ± 0.48	0.1	6.2%
Intraocular Pressure (mmHg)	12.7 ± 3.03	0.1	23.8%

[1]Average ± Standard deviation; [2]Mean Standard Error; [3]Variation Coefficient

Evaluating the degree of linear association between the variables were verified in their assumptions of normality and homoscedasticity and, in the case of meeting the assumptions, correlation studies were established (Pearson's linear correlation coefficient). Among these, significant (P<0.05) and moderate values of negative correlations were observed, when the age and STT variables (-0.485) were contrasted. The results showed a downward trend for STT values as age increases (Figure 2). No other correlations were found between the other variables and the age trend.

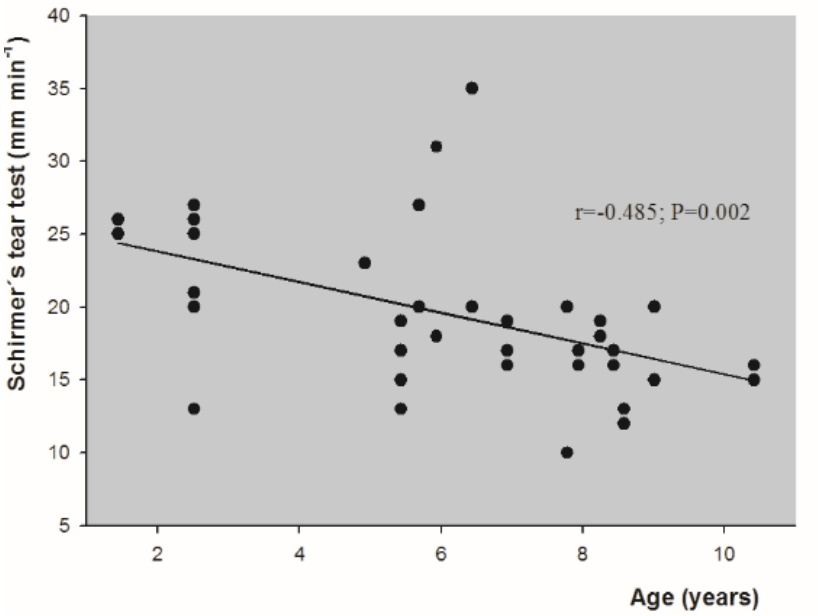

Figure 2 - Pearson correlation diagrams between the age of the sheep and the results for the STT.

After the induction of mydriasis, no alteration in the lens was observed. In indirect ophthalmoscopy, the triangular-shaped tapetal area was observed in the dorsotemporal quadrant. Fundus it was possible to visualize the tapetal area with colours between blue, green and yellow tones and the retinal capillaries (arterioles and venules) highlighting the larger dorsal vessels, surrounding each other. In some animals, discrete blackish focal areas surrounded by a yellowish area (Stars of Winslow) were observed. The transition to the non-tapetal area was observed horizontally, with heterogeneous brown tones predominating, with the presence of vessels in the non-tapetal area. The optical disc was observed predominantly in the tapetal transition area, with an elliptical shape with discreet pigmented edges with holoangiotic retinal vascular.

In terms of the fluorescein test performed on the ocular surface, there was no observation of dye penetration into the cornea or conjunctiva in all tested animals.

DISCUSSION

Knowledge of the normal intervals for ophthalmological exams is essential for the correct diagnosis of eye disorders in different species (Ofri et al., 2002; Oriá et al., 2015).

The STT is widely used in humans and animals' ophthalmology as a way of assessing tear production, promoting the quantitative exploration of the aqueous phase of the tear film (Williams, 2005). Our study comprised the evaluation of healthy and female ewes. Akgül et al. (2017) in their study, did not were observe a statistically significant difference in the STT, between sexes and between the ratio of healthy sheep and sheep with infectious keratoconjunctivitis. The comparison of STT between the right and the left eye showed no significant differences. This finding is in agreement with those reported in other sheep (Ghaffari et al., 2011; Akgül et al., 2017; Dedousi et al., 2019).

Dedousi et al. (2019) in their study of sheep in Greece, observed that STT was significantly affected by the season of the year. Thus, the mean STT values during the summer (August, 20.08 ± 0.66 mm/min) were significantly higher than the values in the winter (February, 15.10 ± 0.53 mm/min). Our study took place in the autumn season (October) and the mean value obtained for the STT in Alentejo sheep was 19.0 ± 5.22 mm/min. In the study by Ghaffari et al. (2011), the average values of the tear test were 18.52 ± 2.55 mm/min with a range between 13.5 and 22 mm/min. Other results showed higher values for the STT for ewes with 26.40 ± 17.70 mm/min (Wieser et al., 2013). In our study, it showed a reduction in tear production due to the increase in the animal's age. İşler et al. (2013), it was noted that the average tear volume of young sheep was lower than in adult animals. Ribeiro et al. (2010) observed that the tear production in Saanen goats increases significantly up to 180 days of age.

The importance of pH as a protective barrier is described in veterinary and human medicine in various organs (Beckwith-Cohen et al., 2014). Tear alkalinization is related to many different eye diseases in humans, such as nasolacrimal duct stenosis, keratoconjunctivitis sicca, and mycotic keratitis (Norn, 1988). Being the population of our study included only systemically and ophthalmologically healthy sheep.

Veterinarians often prescribe ophthalmic medicines that are formulated for humans (Beckwith-Cohen et al., 2014). The pH of these medications has a comfort zone ranging from 6.5 to 7.8 (Beckwith-Cohen et al., 2014). The mean value of tear pH obtained in the sheep of our study was 7.7 ± 0.48. Probably the comfort zone of the ocular pH of the Alentejo sheep is similar to the human one. Ophthalmic solutions must be within a comfortable pH for patients, so as not to induce tearing and blink reflexes

(Malmberg and Lupo, 2004). The animals' comfort of using ophthalmic medications should be considered, as the ophthalmic treatments usually require administration of the medications several times a day.

Basal values of tear pH were obtained in dogs (Beckwith-Cohen et al., 2014; Horn et al., 2016), horses (Beckwith-Cohen et al., 2014), mice (Ruiz-Ederra et al., 2009), llamas (Gionfriddo et al., 2000), cattle (Gionfriddo et al., 2000; Beckwith-Cohen et al., 2014) and humans (Chen and Maurice, 1990), however, no previous studies were found with the mean values of tear pH in sheep.

Litmus paper is a readily available test for measuring pH (Connor and Severn, 2009; Monaghan et al., 2020), being used as a control test to assess tear pH (Connor and Severn, 2009). This method proved to be easy to apply and effective in measuring the lacrimal pH of sheep.

Errors in litmus paper pH measurement can occur because of the difficulty in matching the paper with a scale of colours. These inaccuracies may be related to a small tear film sample that can create difficulty in colour matching, whereas a large amount of sample can wash away pigment from the litmus paper and still dry the paper which will produce a darker colour staining (Connor and Severn, 2009). The variability between users can also create inaccuracies in the reading (Monaghan et al., 2020). To avoid execution and interpretation errors, the contact time with the litmus paper with the tear was standardized and the reading was performed in sequence. In addition, the same clinician performed all the measurements and interpretations of the tests, to reduce subjectivity bias, and there was no report of any difficulty in interpreting the scale of colours.

The mean value obtained for the IOP using the applanation tonometer in the Alentejo sheep was 12.7 ± 3.03 mmHg. Previous studies have shown mean baseline IOP values of 10.6 ± 1.4 mmHg (Gerometta et al., 2009) and 9.6 ± 0.1 mmHg (Gerometta et al., 2010). Other studies of the IOP of healthy sheep showed that the average values were 9.37 ± 2.45 mmHg (Ghaffari et al., 2011) and 16.36 ± 2.19 mmHg (Pigatto et al., 2011). The average normal values of 20.40 ± 3.53 mmHg, were obtained for the IOP in sheep between 2 and 2.5 years old (İşler et al., 2013). No correlation was found in this study between the IOP variable and the age variable. Kulualp et al. (2018) in his study with Awassi sheep, showed that the average of IOP measurements in the morning (16.21 mmHg) and night (12.65 mmHg), decreased by a rate of 22%. In our study, all measurements were made between 9:00 and 12:00 a.m. The animals studied were females, showing that the sex of the animal does no influence on IOP values (İşler et al.,

2013; Kulualp et al., 2018). However, in some species such as lions (Ofri et al., 1998) and humans, the variable gender can influence the IOP values (Pointer, 2000).

Three instrumental tonometry techniques are used in veterinary medicine: indentation, applanation, and rebound tonometry (Gelatt et al., 2021). Applanation tonometry was used in the measurements of Alentejo sheep. A study comparing IOP in sheep using rebound tonometry and applanation tonometry revealed an average pressure of 11mmHg and 10mmHg, respectively (Peche and Eule, 2018). The applanation tonometer is a widely used instrument to estimate IOP in many species and it is considered an accurate and reliable method to estimate this parameter in animals (Rusanen et al., 2010).

In order to avoid variation in the data collection, the examinations were performed by the same professional (Gelatt and Mackay, 1998). In addition, during the physical restraint of the animals, any application of pressure to the neck and head of the animals was avoided, and the head was kept in a position that was higher than the sternum, in order to avoid iatrogenic changes in the IOP (Pigatto et al., 2011). During the exams the animals were kept in pens, with the individuals from the same flock being all together, aiming to reduce stress, alter their behaviour or variations in the values and results (Gelatt and Mackay, 1998).

The the sheep stall made possible the light control, suitable for ophthalmological tests and the observation of ophthalmoscopic characteristics. The patterns that were visualized in the ocular fundus were the ones expected for this specie, observing the tapetal area in the dorsotemporal quadrant, with color tones between green and blue with the presence of capillaries (Galán et al., 2006). The horizontal transition to the non-tapetal area had a predominance of shades of brown, with the presence of vessels in the non-tapetal area (Galán et al., 2006). The optical disc was observed predominantly in the transition area of tapetal and non-tapetal regions of elliptical shape with discreetly pigmented edges with holoangiotic pattern vessels (Galán et al., 2006; Gelatt et al., 2021).

Cobalt blue filter from the slit-lamp biomicroscope was used to exacerbate the fluorescein dye and verify the integrity of the ocular surface. The fluorescein molecule when illuminated with blue light is excited and emits yellow-green light (Tsang and Sharma, 2018). This fluorescence emission facilitates the visualization and identification of areas with dye penetration on the ocular surface. Fluorescein is highly lipophobic and hydrophilic. Not penetrating intact lipid-containing cell membranes of the epithelium

(conjunctival and corneal), but adheres to, and is absorbed by, any posed hydrophilic substance, and therefore assists in the detection of corneal erosions (Gelatt et al., 2021).

Topical fluorescein is used to detect corneal ulceration (most common use), in addition to being used to detect conjunctival defects, aqueous humor leakage, the physiologic flow of the nasolacrimal system, and qualitative tear film abnormalities (Gelatt et al., 2021). The fluorescein test was used only to confirm the absence of lesions on the ocular surface. In addition, the presence of corneal lesions can stimulate increased tear reflex production and reduced IOP (Williams and Burg, 2017), inducing an erroneous interpretation of normality parameters in the reading of the STT and IOP. In this research, these normality parameters may be useful to improve the clinical ophthalmological approach to the studied specie and to provide new reference values for the exams that were tested.

CONCLUSION

The results presented for Alentejo Merino sheep, can be used as references for the STT, IOP and tear pH tests. The lacrimal production evaluated by STT decreases as the increased age the animal. Supplementary investigations in this species are also considered, knowing that eye studies in sheep are still scarce.

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