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Comparative evaluations of tear secretion in healthy and infectious keratoconjunctivitis Romanov sheep with Schirmer tear test and phenol red thread tear test

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Abstract: The aim of this study is to determine the amount of tear secretion in sheep with infectious keratoconjunctivitis and healthy ones in the Siirt region by Schirmer tear test and phenol red thread tear test. A total of 47 Romanov sheep, consisting of sheep with infectious keratoconjunctivitis (n = 6) and healthy sheep (n = 41), constituted the study material. Phenol red thread tear test followed by Schirmer tear test was performed on both eyes of the animals, properly taken at a control visit on their feet. The Schirmer tear test and phenol red thread tear test values were measured as 18.83 ± 5.29 mm/min and 22.22 ± 6.09 mm/s in the right eyes and 18.41 ± 7.15 mm/min and 20.83 ± 5.72 mm/s in the left eyes, respectively, in healthy animals. The Schirmer tear test and phenol red thread tear test values were measured as 17.33 ± 4.27 mm/min and 18.83 ± 4.36 mm/s in the right eyes and 17.33 ± 7.55 mm/min and 20.17 ± 4.58 mm/s in the left eyes, respectively, in animals with infectious keratoconjunctivitis. The phenol red thread tear test measurement averages were found to be significantly higher than the Schirmer tear test measurement averages ($P > 0.05$). The amount of normal tear secretion was determined in Romanov breed sheep by the phenol red thread tear test and Schirmer tear test and there was no statistically significant difference in animals with infectious keratoconjunctivitis ($P > 0.05$).

Key words: Eye, sheep, ophthalmic examination, tear production, Schirmer tear test

1. Introduction

Ocular tears are formed by the secretion of many glands (1–5). The outer lipid layer is produced by Meibomian and Zeis glands located in the upper and lower eyelids. The middle layer is produced by the orbital tear gland and the third eyelid gland. The innermost mucoprotein layer is generated by the conjunctival goblet and corneal epithelial cells (6–10).

The spread of tears on the eye occurs as a result of the movements of the third, lower, and upper eyelids (11). Tears are necessary to maintain normal function of the conjunctiva and cornea. Tears play an important role in removing foreign substances from the eye, provide the necessary nutrient content for the avascular cornea, and contain immunoglobulin and lysosomes, which are significant for the defense mechanism of the eye (1).

The Schirmer tear test (STT) and phenol red thread tear test (PRT) are utilized to determine the amount of tear secretion in different strains, the break-up time (BUT) test is used for stability of precorneal tear film, and dyeing tests such as rose Bengal, lissamine green, and fluorescein are used to control the integrity of the precorneal tear film (12). The idea of using a thread to measure the amount of tears was originally conceived by Kurihashi et al. in 1975 (13). Hamano et al. introduced the PRT test in 1982 (14).

The PRT employs phenol red, a pH indicator, and an impregnated strip thread that is 75 mm long (15,16). The STT was introduced over a century ago and is still widely used in clinical practice (17). Separately packed sterile test strips consist of number 41 Whatman filter paper, graded 5 mm wide, 5 mm spaced, and 50 mm long, with a notch of 5 mm each (18).

Infectious keratoconjunctivitis of sheep is encountered in many parts of the world. Also known as 'pink eye', it is an economically important and contagious disease of small ruminants (19–22). Emergence of clinical findings may begin unilaterally but is mostly seen bilaterally (22,23). The first indications of the disease are conjunctival hyperemia, serous lacrimation, increased blinking, photophobia, and blepharospasm (22,24). Keratitis and corneal ulcers develop in later stages of the disease and may lead to permanent visual loss (22,25,26). Although many

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different microorganisms are mentioned in the etiology of the disease, the causes and predisposing factors of the disease continue to be investigated today. *Branhamella ovis*, *Chlamydia psittaci*, and *Mycoplasma conjunctiva* are demonstrated as the main known factors (26,27).

The aim of this study is to comparatively evaluate the tear secretion in healthy sheep and sheep with infectious keratoconjunctivitis, of the Romanov breed, raised in the Siirt region by utilizing STT and PRT.

2. Materials and methods

2.1. Ethical committee

This study was approved by the Siirt University Animal Experiments Local Ethics Committee (HADYEK), decision no. 2016/02/14, and was carried out at the Siirt University Goat Research and Application Center (KEÇİMER).

2.2. Animals material and selection

The study material consists of a total of 47 Romanov breed sheep, some with infected keratoconjunctivitis (n = 6, female) and some healthy (n = 41, female and male), housed under the same care and nutrition conditions between the ages of 1 and 1.5 years old at KEÇİMER.

Animals with conjunctival hyperemia, blepharospasm, photophobia, purulent lacrimation, and varying degrees of corneal opacity were evaluated as having infectious keratoconjunctivitis and included in the study.

2.3. Implementing of tests

The animals were placed in a closed and low-light area for the application of the tests. Applications were started at 1000 hours and ambient temperature was measured at 30 °C by a digital thermometer. Tests were carried out by the same researcher, first on the right-hand side and then on the left-hand side of the test subject, at control visits while animals were on their feet. The results were processed into a prepared chart. The first applied test to both eyes was the PRT (PRT-Test, JM®, China). The strip was inserted into the lateral cannula of the lower conjunctival fornix for 15 s after bending 3 mm from the end of the thread. Then the thread dyed in red was measured with the graded part on the package and recorded. After completion of measurements for all subjects, the STT (Schirmer Tear Test, ERC, Turkey) was applied secondly based on the same order of test subjects. The test strip was placed towards the test subject's lower fornix through the middle third of the eye and the outer third of the eye by folding it about 5 mm from its upper end. At the end of 1 min of waiting time, the test was performed and the numerical value was recorded. The same procedure was repeated for the other eye.

2.4. Microbiological analysis

Sterilized eye swabs from the right and left eyes of the controls and the animals with the disease were sent to the Uludağ University Veterinary Faculty's Microbiology Department with cold chain in Cary Blair transport medium. Fed swabs in blood agar, MacConkey agar, eosin methylene blue agar, pathogenic fungi, and *Mycoplasma* agar were incubated for 24–96 h in both aerobic and microaerophilic media. The resulting colonies with different macroscopic morphologies were reapplied by separating them into new-blooded agar and Gram staining of breeding colonies was performed. After examination of the microscopic morphology of the colonies, biochemical tests were performed according to the suspected factors.

2.5. Statistical analysis

Prior to the significance tests, the variables were examined by Shapiro–Wilk test in terms of normality from the parametric test assumptions and by Levene test in terms of the homogeneity of variances. The Student t-test was used to assess the significance of differences between measurements made in terms of sex and health status. A paired sample t-test was used to evaluate the difference between left and right eye measurements of the STT and PRT. The $P > 0.05$ criterion was used for all results. SPSS 14.01 was utilized for statistical analysis (SPSS Inc., Chicago, IL, USA).

3. Results

There was no significant difference between sexes in terms of the STT and PRT values for both right and left eye measurements ($P > 0.05$). The STT values were measured in healthy animals as 18.83 ± 5.29 mm/min in the right eye and 18.41 ± 7.15 mm/min in the left eye (Table 1). There was no statistically significant difference between right and left eye measurements in the STT test results in the control group ($P > 0.05$). The PRT values were measured as 22.22 ± 6.09 mm/s and 20.83 ± 5.72 mm/s in the right eye and in the left eye, respectively, in healthy animals. There was no statistically significant difference between right and left eye measurements in the PRT ($P > 0.05$).

The STT values were measured in infectious keratoconjunctivitis cases as 17.33 ± 4.27 mm/min in the right eye and 17.33 ± 7.55 mm/min in the left eye. The PRT values were measured as 18.83 ± 4.36 mm/s and

Table 1. Reference values of tear production from the healthy eyes as determined by Schirmer tear test strips and phenol red thread tear test.

Tests		N	Mean	SD
STT, mm/min	Right	41	18.83	5.29
	Left	41	18.41	7.15
PRT, mm/s	Right	41	22.22	6.09
	Left	41	20.83	5.72

Table 2. STT and PRT test values between infectious keratoconjunctivitis (IKC) and healthy animals.

Tests	IKC				Healthy			
		N	Mean	SD	N	Mean	SD	P
STT	Right	6	17.33	4.27	41	18.83	5.29	0.512
	Left	6	17.33	7.55	41	18.41	7.15	0.733
PRT	Right	6	18.83	4.36	41	22.22	6.09	0.197
	Left	6	20.17	4.58	41	20.83	5.72	0.788

STT, mm/min; PRT, mm/s.

20.17 ± 4.58 mm/s in the right eye and in the left eye, respectively, in infectious keratoconjunctivitis-infected animals. There was no significant difference between infectious keratoconjunctivitis cases and healthy animals in terms of the STT and PRT values ($P > 0.05$) (Table 2). The averages of the PRT measurements were found to be significantly higher than the STT measurement averages in all of the healthy animals ($P > 0.05$). *Staphylococcus aureus* sp., *Clostridium* spp., and *Penicillium* spp. were isolated as a result of Gram stains and biochemical tests of breeding colonies in swabs collected from the eyes of sick animals ($n = 6$) when no growth was detected in the swabs of the control group.

4. Discussion

The STT, designed by Otto Schirmer a century ago, has been widely used as a basic evaluation method of tear production in both human and veterinary ophthalmology (28).

The PRT was designed to measure the amount of tears remaining in the lower conjunctival sac and it was developed to overcome the disadvantages of the STT including variable results, poor repeatability, and low sensitivity in detecting dry eyes (29).

Although the time required for the STT is 1 min per eye, only 15 s of testing time is required for the PRT. The PRT does not cause reflex tear production as it causes minimal discomfort to the eye (29). First, the application of the PRT provided a better chance of making a more accurate assessment of the STT by preventing an increase in reflex tear production in animals. In addition, the ability to perform the PRT in as little as 15 s caused less stress to animals compared to the STT. Reduction of the functional capacity of the lacrimal and nictitans glands with age is thought to cause a reduction in tear production. Although it has been found that there is a similar relationship between age and tear production in animals and people, some studies have discussed that there is no relationship between tear production and age, sex, or weight (30,31).

STT values were found to be lower in female dogs; therefore, keratoconjunctivitis sicca may have a high incidence in females (32). There was no difference seen between left and right eyes in the same age group of both male and female sheep.

It has been reported that diseases such as chronic eye infections, conjunctivitis, and keratoconjunctivitis that cause damage to lacrimal glands in dogs cause a permanent or temporary decrease in tear secretion. In the sheep, there was no statistically significant difference between the diseased and the healthy subjects in terms of STT and PRT values ($P > 0.05$) (3).

There are large differences between species in normal STT values. Normal tear secretion was reported as 15.1 mm/min in rhesus monkey (*Macaca mulatta*), 24.9 mm/min in the African lion (*Panthera leo*), 13.2 mm/min in Nubian goat (*Capra ibex nubiana*), 23.4 mm/min in Burchell's zebra (*Equus burchelli*), 10.2 mm/min in the Arabian oryx (*Oryx leucoryx*), 16.9 mm/min in cats, 14–24 mm/min in dogs, and 18.52 ± 2.55 mm/min in Sanjabi sheep (11,33–38). STT values were measured as 18.83 ± 5.29 mm/min in the right eye and 18.41 ± 7.15 mm/min in the left eye in Romanov breed animals. Tear measurements were performed in dogs, cats, horses, and many other animal species by the PRT (15). However, although there are measurements of the amount of tears using the STT in sheep, sufficient scientific data on tear secretion by the PRT could not be found. In this study, the PRT values were measured in healthy animals as 22.22 ± 6.09 mm/s in the right eye and 20.83 ± 5.72 mm/s in the left eye. There was no statistically significant difference between right and left eye measurements in the PRT results ($P > 0.05$).

Cakir et al. (39) isolated and identified *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* in eye swabs obtained from 30 Saanen breed goats with keratoconjunctivitis. Åkerstedt et al. (40) identified *Bacillus* spp., *Corynebacterium* spp., *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus* spp., *Moraxella* (*Branhamella*) *ovis*, *Moraxella* spp., *Mycoplasma conjunctivae*, *Pseudomonas* spp., *Staphylococcus aureus*,

Staphylococcus spp., and *Streptococcus* spp. in isolation from 135 Norway breed bovine keratoconjunctivitis infections. In our study, *Staphylococcus aureus* sp., *Clostridium* spp., and *Penicillium* spp. were isolated from sheep with infectious keratoconjunctivitis.

As a result of this study, the amount of normal tear secretion was determined in Romanov sheep with the PRT and STT and there was no statistically significant difference between these two tests with infectious keratoconjunctivitis-infected animals ($P > 0.05$). The PRT has been shown to be more advantageous than the STT as it is much quicker to perform and easier to apply to sheep. Although there was no statistically significant difference between females and males or right and left eyes, the mean of the PRT measurements was found to be significantly higher than the mean of the STT measurements ($P > 0.05$).

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