

## EVALUATIONS OF TEAR SECRETION IN AN OUTBREAK OF ESCHERICHIA COLI IN LAMBS WITH SCHIRMER TEAR TEST

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### ABSTRACT

This study aims to determinate the tear secretion in lambs with colibacillosis and healthy ones by utilizing Schirmer Tear Test. A total of 25 lambs aged between 1-6 days, among which 15 were with *Escherichia coli* (Group 1, n=15) and 10 were in the healthy conditions (Group 2, n=10). The lambs in the study group were diagnosed for *Escherichia coli* by using a sheep-specific rapid diagnosis kit before being included in the group. Lambs to be included in the control group were selected from among the lambs that did not show any signs of disease and never had diarrhea after birth. Schirmer Tear Test was performed on both eyes of healthy and *Escherichia coli* and results were recorded. Group 1 Mean  $\pm$  SD Schirmer Tear Test values for right and left eye were  $6.47 \pm 1.55$  and  $7.33 \pm 1.45$  mm/min and Group 2 Mean  $\pm$  SD Schirmer Tear Test values for right and left eye were  $15.7 \pm 9.07$  and  $17.5 \pm 8.36$  mm/min, respectively. Comparison between Schirmer Tear Test values of right eyes and left eyes revealed no significant differences in either of groups ( $P=0.063$ ,  $P=0.306$ ). Both eye's Schirmer Tear Test results were combined to compare groups. The mean of Group 1 ( $6.9 \pm 1.54$ ) was found to be significantly lower than the mean of Group 2 ( $16.6 \pm 8.54$ ) as a result ( $P < 0.001$ ). As a result of this study, the amount of tear secretion was determined with the Schirmer Tear Test and there was a statistically significant difference between healthy and lambs with *Escherichia coli*.

**Key Words:** *Escherichia coli*, Schirmer Tear Test, Tear.

### INTRODUCTION

Colibacillosis is a disease course affecting ruminants characterized by neonatal diarrhea caused by different pathogenic stereotypes of *Escherichia coli*.

*Escherichia coli*, which is taxonomically classified in the Enterobacteriaceae family, is a bacterial species that can survive in the large intestinal flora of mammals and poultry (Şahin and Başaoğlu, 2011). It is possible to discuss different categories of colibacillosis caused by different stereotypes such as enterotoxigenic (ETEC), enteropathogenic, enterohemorrhagic, necrotoxic and septicemic. Strains of several stereotypes of *Escherichia coli* cause diarrhea in lambs. Pili such as K 99 (F5), F

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41, F (Y) and Att 25 and the thermostable enterotoxins they synthesize are of great importance for *Escherichia coli* strains that are pathogenic for lambs. Failure of passive transfer (FPT), starvation, cold weather conditions and the presence of other viral and parasitic factors contribute to the emergence of the disease. Hypersecretion resulting from bacterial attachment to enterocytes causes diarrhea. Bicarbonate and fluid loss caused by diarrhea result in severe dehydration and metabolic acidosis (Rodostitis *et al.*, 2004). *Escherichia coli* is also common in water and soil due to its ability to survive in non-intestinal environments (Şahin and Başaoğlu, 2011).

Colibacillosis is a serious problem that causes economic losses in many countries. Intestinal diseases caused by *Escherichia coli* strains, which usually manifest as diarrhea, may further turn into diarrhea cases that cause heavy fluid losses (Şahin, 2005; Cerna-Cortes *et al.*, 2013). Diarrhea caused by colibacillosis seen in calves is also frequently observed in lambs and kids (Cid *et al.*, 1996). A study by Sharif and Obeidat (2005) reported the rate of diarrhea cases resulting in death in 242 lambs and kids from different herds as 59.75%. It was further stated in the same study that 63.4% of the bacteria detected were *Escherichia coli*. In another study, it was determined that 48% of diarrhea cases observed in lambs were caused by *Escherichia coli* (Garcia *et al.*, 2000).

For the diagnosis of *Escherichia coli*, it is necessary to detect K99 and F41 fimbria antigen in Ovine ETEC isolates. Diagnosis is made by examining the fimbriae antigen of *Escherichia coli* in smears performed on stool samples collected particularly from the ileum. In addition, rapid diagnostic test kits used in field studies were also used due to their practicality (Rodostitis *et al.*, 2004).

Severe complications due to lamb diarrhea, which is frequently encountered in the first four weeks after birth in lambs, may cause

death or growth retardation. *Escherichia coli*, rotavirus, coronavirus and cryptosporidium species are reported as the most common causes of lamb's diarrhea. When examining lambs' diarrhea cases, it is seen that hypersecretion in the digestive system prevents reabsorption of intestinal contents (malresorption) which thereby triggers the buffering mechanism causing fluid accumulation in the lumen and consequent diarrhea. This is the case observed in the increased intestinal secretions and in the acute inflammatory conditions. Different degrees of dehydration are observed depending on the severity and duration of diarrhea. Dehydration caused by fluid loss in neonatal lamb diarrhea cases results in hemoconcentration of the blood. This causes a decrease in plasma volume and insufficient perfusion of tissues (Tennant *et al.*, 1972).

One of the clinical signs of dehydration is decreased tear secretion. The Schirmer Tear Test (STT), which helps to determine whether the eye produces enough tears, is measured by two methods. Through STT-I, no local anesthetic is applied to the eye and the amount of tears absorbed by the test strip within 1 minute is taken as a criterion. The aim is to measure both the basal tear level and the reflex tear amount. Through STT-II, a local anesthetic is dripped into the eye to be measured and the measurement is made after the drug has taken effect. Reflex tear amount cannot be measured in this test as a local anesthetic is dripped into the eye. The measurement value is approximately 80% of STT-I (Şaroğlu, 2013).

In the literature review, no research was found that examined the amount of tears secreted in lamb diarrhea caused by *Escherichia coli*. This study aims to compare the amount of secreted tears by healthy lambs and *Escherichia coli*-diagnosed lambs using the Schirmer Tear Test (STT-1), to close the information gap in the literature with the data obtained and to shed light on future studies.

## MATERIALS AND METODS

### Animal Material

The study was conducted with 25 lambs aged between 1-6 days, among which 15 were with *Escherichia coli* (Group 1, n=15) and 10 were in the healthy conditions (Group 2, n=10), in a single farm located at Siirt, Turkey. Housing, feeding, and management conditions were the same for all lambs. The clinical examinations (body temperature (°C), respiratory rate (/min), heart rate (/min), sucking reflex score (between 1-3), stool score (between 1-3), mental status (1-3) foam in the mouth and/or lesion, nasal and/or tear discharge, cough, joint swelling, nervous findings and abdominal pain) of all animals in the study were extensively performed. The lambs in the study group were diagnosed for *Escherichia coli* by using a sheep-specific rapid diagnosis kit before being included in the group. Lambs to be included in the control group were selected from among the lambs that did not show any signs of disease and never had diarrhea after birth. Lambs with clinical signs and symptoms related to the infection of a different system (respiratory infections, omphalophlebitis, orthopedic problems, etc.) were not included in the study.

### Collection of the Samples

Stool samples were collected by rectal touches or waiting for spontaneous defecation in the stool sample container (Polypropylene Sterile Sample Cup, Firat, Turkey) for 30 gr each. *Escherichia coli* was identified as the etiological factor using the rapid ELISA kit (qualitative analysis by immunochromatographic method; sensitivity and specificity rates 98% and 99%).

### Implementation of test:

The animals were placed in a closed and low-light area for the application of the Schirmer Tear Test (Schirmer Tear Test, ERC, Turkey). 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. 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.

### Statistical analysis:

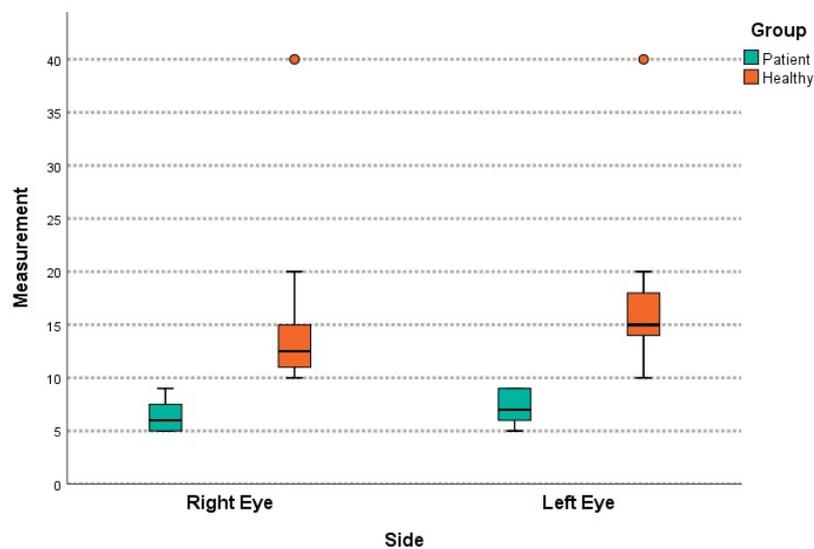
An inspection of Q-Q plot suggested that the assumption of normality was violated. In line with this, Shapiro-Wilk test also suggested that Schirmer Tear Test values were not normally distributed. A Wilcoxon signed-rank test was used to determine the difference between right and left eye tear secretions in each group individually. A Mann-Whitney U test was conducted to reveal the difference between patient and healthy groups. Results were summarized as Mean±Standart Deviation (SD) and Median [Lower Quartile (Q1)-Upper Quartile (Q3)]. All statistical analyses were examined with a critical value of  $P < 0.05$ . Data were analyzed by using The Statistical Package for the Social Sciences (SPSS 26.0, IBM) software package.

## RESULTS

Group 1 Mean  $\pm$  SD Schirmer Tear Test values for right and left eye were  $6.47 \pm 1.55$  and  $7.33 \pm 1.45$  mm/min and Group 2 Mean  $\pm$  SD Schirmer Tear Test values for right and left eye were  $15.7 \pm 9.07$  and  $17.5 \pm 8.36$  mm/min, respectively. Comparison between Schirmer Tear Test values of right eyes and left eyes revealed no significant differences in either of the groups ( $P=0.063$ ,  $P=0.306$ ). Therefore, both eye's Schirmer Tear Test results were pooled to compare groups. The mean of Group 1 ( $6.9 \pm 1.54$ ) was found to be significantly lower than the mean of Group 2 ( $16.6 \pm 8.54$ ) as a result ( $P < 0.001$ ) (Table 1, figure 1).

**Table 1:** Mean  $\pm$  SD results as collected from right, left and both eyes.

Group	Side	N	Mean $\pm$ SD	Median (Q <sub>1</sub> -Q <sub>3</sub> )	P Value
Group 1	Right Eye	15	6.47 $\pm$ 1.55	6 (5-7.5)	0.063
	Left Eye	15	7.33 $\pm$ 1.45	7 (6-9)	
Group 2	Right Eye	10	15.7 $\pm$ 9.07	12.5 (11-15)	0.306
	Left Eye	10	17.5 $\pm$ 8.36	15 (14-17.5)	
Group 1	Both Eye	30	6.9 $\pm$ 1.54	6 (6-8.75)	<0.001
Group 2		20	16.6 $\pm$ 8.54	14.5 (11.75-16.5)	

**Figure 1:** Results as collected from right, left and both eyes

## DISCUSSION

Colibacillosis is a lethal group of diseases caused by pathogenic strains of *Escherichia coli*, characterized by enteritis, toxemia and septicemia. *Escherichia coli* causes watery diarrhea, dehydration and thus weakness in small lambs and causes significant economic losses in the context of newborn lambs (Temizel, 2017).

One of the factors affecting diarrhea in neonatal ruminants is *Escherichia coli* K99 (F5) and three different virulence factors, namely fimbriae, fimbrial adhesions and enterotoxin adhering to the intestinal epithelium play a role in the pathogenesis of associated infections (Nataro *et al.*, 1987).

Prior research has revealed that 10.5% of lamb and kid diarrhea is caused by *Escherichia coli* (F5) and 10.9% is caused by *Escherichia coli* O157:H7 strains (Garcia *et al.*, 2000). The rapid test kit used in the present study tests the presence of the *Escherichia coli* K99 (F5) strain.

Veterinary ophthalmology has made great progress in recent years and has turned into a significant industry with the introduction of technological devices into the field and the increased awareness of infected animal owners about ocular lesions. The amount of aqueous tear production in animals is measured using the Schirmer Tear Test (STT- I and II) and Phenol Red Thread Test (PRTT) (Barabino *et al.*, 2004; Biricik *et al.*,

2003; Erol *et al.*, 2018). Tears produced as a result of basal and reflex secretion, depending on the control of the autonomic nervous system, are usually measured with the Schirmer Tear Test. Schirmer Tear Test is known as the most preferred method in large and small animals with wide palpebrae length (Barabino *et al.*, 2004; Zhu *et al.*, 2003). Being easily administered, being affordable and not requiring the animals to be restrained in any way are considered among the advantages of the Schirmer Tear Test (Gelatt, 2012) whereas the low specificity and sensitivity are reported as primary disadvantages (Lange *et al.*, 2013). On the other hand, the Schirmer Tear Test was preferred herein as it is still routinely used to measure the amount of aqueous tear production in animals, as it is easy to administer and as it does not require the animals to be restrained. There were researchers (Martins *et al.*, 2009; Sakamoto *et al.*, 1993) who have provided some reports on the disadvantages (e.g eye irritation) of Schirmer Tear Test strips, however, we encountered no similar complications during and after this procedure provided that the sterile Schirmer Tear Test strip was inserted correctly.

In various studies conducted on healthy animals, the mean STT-I value was reported as  $17.1 \pm 2.8$  mm/min in dogs,  $16.2 \pm 3.8$  mm/min in cats (Koç *et al.*, 2005),  $15.8 \pm 5.7$  mm/min in goats,  $18.5 \pm 2.5$  mm/min in sheep (Trbolova and Ghaffari, 2011) and  $12.72 \pm 9.07$  mm/min in horses (Beech *et al.*, 2003). In line with the data collected herein mean STT-I value of healthy animals was determined as  $15.7 \pm 9.07$  mm/min for the right eye and  $17.5 \pm 8.36$  mm/min for the left eye.

In another study conducted in veterinary medicine, it was reported that head shape or age variables have no effect on the Schirmer Tear Test results of dogs and, even, bodyweight has no effect on tear secretion (Wyman *et al.*, 1995). On the contrary, Berger *et al.* (1998) concluded in their study

that bodyweight affects tear secretion. Furthermore, although a similar relationship between age and tear secretion was found in animals and humans, other studies argued that there is no relationship between tear production and age, gender or body weight (Moss *et al.*, 2004; Mathers *et al.*, 1996). Some researchers argued the opposite and reported that the amount of aqueous tear production in domestic animals is affected by the breed variable and the results obtained differ in accordance with the breeds although the same tests are used (Alkan *et al.*, 2004, Hakanson and Arnesson, 1997; Hamor *et al.*, 2000; Saito and Kotani, 2001). Hartley *et al.* (2006) stated in another study they conducted that tear secretion of dogs decreases as they age. Therefore, lambs of the same breed and with an average weight of 2 kg from the same farm were chosen as the study material of the present study.

Dehydration can have a number of unwanted effects on the body, and the eyes without exception. The eyes need water just as much as joints or kidneys in order to function properly. Dehydration has some symptoms such as dry mouth, decreased urine output, muscle cramps, lightheadedness and a lack of tear production (McCarty *et al.*, 1998). In a study conducted with humans, Köşger *et al.* (2018) concluded that the patient's excessive systemic dehydration contributed to the symptoms and signs of dry eye. The findings in the present study arguing that the STT values of the study group consisting of infected animals ( $6.9 \pm 1.54$ ) were statistically lower compared to the control group ( $16.6 \pm 8.54$ ) suggest that dehydration, which manifests as a result of excessive diarrhea caused by *Escherichia coli*, similarly causes dry eye in patients.

## CONCLUSION

This study aimed to measure the amount of tear secretion in healthy lambs and lambs infected with *Escherichia coli* using the Schirmer Tear Test and concluded that that there is a statistically significant difference

in the amount of tear secretion between healthy and infected lambs. The findings revealed that the amount of tear secretion of the lambs infected with *Escherichia coli* was significantly lower than the healthy ones. We believe in the opinion that the amount of tear secreted decreases as a result of dehydration due to fluid loss caused by clinical symptoms of *Escherichia coli*. This study is the first in its field in terms of the subject it researches and we believe that further studies are required to clarify the factors affecting tear secretion in lambs diagnosed with *Escherichia coli*.

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