EVALUATION OF THE SCHIRMER TEAR TEST IN CLINICALLY NORMAL TURKISH HUNTING DOGS (TURKISH GREYHOUND)

F. Alkan[1], C. Izci[1], C. Tepeli[2] and Y. Koc[1]

[1]Department of Surgery, College of Veterinary Medicine, University of Selcuk, Konya, Turkey
[2]Department of Animal Breeding, College of Veterinary Medicine, University of Selcuk, Konya, Turkey
falkan@selcuk.edu.tr

ABSTRACT

The aim of this study was to determine the normal values for the Schirmer tear test (STT) in Turkish Hunting Dogs. This was performed in order to facilitate the diagnosis of xerophthalmia or keratoconjunctivitis sicca (KCS), which is characterized by deficiencies in tear production and the resulting abnormal STT. Fifteen Turkish Hunting Dogs (8 female and 7 male) were used in this study. The dogs ranged from 6 months to 9 years old (mean 3.6 ±2.8) and the mean weight was 21.3 kg (13 to 27). The mean STT-1 and STT-2 values were 17.1 ±3.7 mm per minute and 7.7 ±2.8 mm per minute, respectively. Neither body weight nor age had a statistically significant effect on these values. There were significant differences in both STT-1 and STT-2 values between females and males ($p<0.05$), as well as daily and weekly fluctuations for STT-1 and STT-2 values ($P<0.01$, $P<0.05$, respectively). This study has shown that the STT-1 and STT-2 values in the Turkish Hunting Dogs are normally distributed and are significantly affected by sex and by measurement sequences.

INTRODUCTION

The preocular tear film (PTF) is composed of three layers and is about 7µ in thickness (Severin, 1986; Slatter, 1990; Aguirre *et al.*, 1995). The outermost lipid component is produced by the Meibomian glands in the upper and lower eyelids. The middle aqueous component is produced by the lacrimal gland and the gland of the nictitating membrane. The inner mucin component is produced by conjunctival goblet cells and epithelia of the ocular surface (Severin, 1986; Whitley *et al.*, 1991; Alkan *et al.*, 2004). The distribution of PTF occurs as a result of the movements of the upper and lower eyelids and the third eyelid. The components of the PTF are responsible for maintaining the health and normal function of the ocular surface. Tears help to remove waste products and debris, they provide moisture, lubrication, and essential nutrients (oxygen and glucose) to the avascular cornea, and they contain immunoglobulins, enzymes, and other proteins which are important for protecting the eye (Slatter, 1990; Wilkie, 1993 Izci *et al.*, 2002).

Deficiencies in the PTF secretion result in an inflammation of the conjunctiva and cornea which in dogs is known as xerophthalmia or keratoconjunctivitis sicca (KCS) (Kaswan and Salisbury, 1990; Whitley *et al.*, 1991; Izci *et al.*, 1995). Some breeds of dogs, such as the American Cocker Spaniel, Miniature Schnauzer, Poodle, Shih Tzu, Lhasa Apso, Pug and English Bulldog, are predisposed to this disease due to genetic factors (Severin, 1986; Slatter, 1990; Wilkie, 1993; Izci *et al.*, 2002). The condition is characterized by a deficiency in the aqueous portion of the PTF. The hallmark clinical signs include mucoid discharge, blepharospasm, conjunctival hyperemia and chemosis, in addition to corneal ulceration, vascularization and pigmention (Kaswan and Salisbury, 1990; Wilkie, 1993; Aguirre *et al.*, 1995).

The diagnosis of KCS or xerophthalmia is usually made on the basis of the clinical signs and confirmed by the Schirmer tear test (STT). Normal STT values in healthy dogs tend to cluster between 14 and 24 mm wetting/minute (Table 1) (Rubin *et al.*, 1965; Gelatt *et al.*, 1975; Berger and King, 1998; Alkan *et al.*, 2004). Dogs with 8 to 14 mm wetting per minute may have KCS (Severin, 1986; Wilkie, 1993; Aguirre *et al.*, 1995; Izci *et al.*, 1995). In the presence of typical clinical signs, any figure below 5 mm wetting per minute is regarded as abnormal and diagnostic for KCS (Kaswan and Salisbury, 1990; Slatter, 1990; Izci *et al.*, 2002). However, significant differences are found in Schirmer tear test values between dogs of different breeds (Hakanson and Arnesson, 1997; Hamor *et al.*, 2000; Saito and Kotani, 2001; Alkan *et al.*, 2004).

Table 1. The mean values of STT-1 and STT-2 in clinically normal dogs of seven breeds.

Breed	STT-1 (mm/min)	STT-2 (mm/min)
Beagle	18.9 ± 2.6	9.5 ± 4.5
Labrador Retriever	22.9 ± 4.1	9.6 ± 3.8
English Springer Spaniel	20.7 ± 3.2	5.4 ± 3.4
Golden Retriever	21.8 ± 3.7	8.8 ± 3.1
Shetland Sheepdog	15.8 ± 1.8	3.6 ± 2.8
Turkish Shepherd Dog	21.1 ± 3.4	9.8 ± 2.7
Shih Tzu	19.5 ± 4.1	9.2 ± 4.5

Data from Rubin *et al.*; 1965, Saito and Kotani, 2001; Hamor *et al.*, 2000; Alkan *et al.*, 2004.

Thus, what is considered normal tear production in one breed could be regarded as deficient in another. Therefore, it is very difficult to rule out KCS as a differential diagnosis for different breeds in which normal values are not known (Saito and Kotani, 1999; Hamor *et al.*, 2000; Alkan *et al.*, 2004).

For a number of canine breeds, STT values are documented in the literature (Table 1), but very little is known about STT values in Greyhounds (Rubin *et al.*, 1965; Wyman *et al.*, 1995; Berger and King, 1998). Moreover, Turkish Hunting Dogs with KCS were encountered recently and the STT was widely used in diagnosis and monitoring response to treatment. However, published data on normal STT values in this breed are absent. The aims of this study are (1) to determine the normal values for the Schirmer tear test (STT-1) and modified Schirmer tear test (STT-2) with topical anesthesia in Turkish Hunting Dogs, (2) to determine whether the age and sex of the dogs have any effect upon the Schirmer values, (3) to determine whether there are significant daily or weekly fluctuations in STT values, and (4) to identify the variables that might influence these findings.

MATERIALS AND METHODS

Animals

Fifteen Turkish Hunting Dogs (Turkish Greyhounds) reared at the Veterinary Faculty Farm of Selcuk University were used in this study. All the dogs were given a general physical and ocular examination to rule out pre-existing ocular or physical abnormalities. Age, body weight and sex were recorded for each dog. They were housed indoors at a room temperature of 17 ±2.3º C and a humidity of 55 ±10%, and were fed once a day between 10 am and noon.

Experimental design

The experimental protocol was approved by the managerial board of the animal farm of the Veterinary Faculty at the University of Selcuk in Turkey. Schirmer tear testing paper (Clement Clarke International Limited) was used for the Schirmer tests. The STT-1 was measured by inserting a bent portion of the test paper into the anterior medial one-third of the inferior conjunctival sac. The test paper was removed after the eyelids had been closed for 60 seconds and the length of the wet portion of the test paper was measured in mm. For STT-2, one drop of 0.4 oxybuprocaine hydrochloride (Benoxinate % 0.4 Thilo, Liba A.S) was instilled on the surface of the eye, followed by a second drop 30 seconds later. After 5 minutes, residual tear in the conjunctival sac was gently dried with a cotton-tipped swab (Soffio, Sogedv, Brascia) and the STT-2 was performed in the same manner as described for the STT-1 (Rubin *et al.*, 1965; Gelatt *et al.*, 1975; Slatter, 1990; Hakanson and Arnesson, 1997; Hamor *et al.*, 2000). The STT-1 and STT-2 measurements were performed twice a day, viz. between 8 and 10 am and again between 3 and 5 pm., for seven consecutive days and again on the 14th, 21st and 28th days on both eyes of all the dogs. All tests were performed by

Table 2. Results of STT-1 and STT-2 in Turkish Hunting Dogs (mm/per minute).

Days	STT-1	STT-2
Day 1	18.9 ± 3.4[ab]	7.7±2.3[ab]
Day 2	16.0 ± 3.5[abc]	7.2±3.1[ab]
Day 3	15.6 ± 2.8[bc]	6.1±2.3[b]
Day 4	20.5 ± 3.1[a]	9.7±1.9[a]
Day 5	14.3 ± 2.8[c]	6.3±2.3[ab]
Day 6	16.9 ± 3.4[abc]	6.6±2.3[ab]
Day 7	18.8 ± 3.7[ab]	9.3±3.0[a]
Day 14	16.6 ±4.0[abc]	7.7±3.1[ab]
Day 21	15.5 ± 2.8[bc]	6.3±2.6[ab]
Day 28	18.2 ± 3.2[abc]	9.7±2.6[a]
Mean values	17.1 ± 3.7	7.7±2.8

[abc] Values in the same column with different superscripts are statistically significantly different (P<0.05, P<0.01)
Data expressed as mean ± SEM mm wetting per minute.

Table 3. Results of STT-1 and STT-2 of different age, sex and daytime groups in Turkish Hunting Dogs (mm/per minute).

Groups	STTT-1	STT-2
Age		
Young	16.2±3.3	7.7±2.2
Middle	16.6±3.5	7.1±2.9
Old	18.3±4.3	8.5±3.3
Sex		
Female	17.9± 3.4 [a]	8.3±2.4 [a]
Male	16.3±3.7 [b]	7.2±3.0 [b]
Daytime		
Morning	16.4 ± 3.6 [b]	7.0 ± 2.9 [b]
Afternoon	17.8 ± 4.2 [a]	8.3 ± 3.3 [a]

[ab]Values in the same column with different superscripts are statistically significantly different (P<0.05).
Data expressed as mean ± SEM. mm wetting per minute.

the same persons. For data analyses, the dogs were assigned to one of three age categories: young (less than two years), middle-aged (three to five years), and old (older than five years).

Statistical analyses

The experimental analyses of the data were carried out by one-way analysis of variance (ANOVA). The differences between groups were compared using Duncan's Multiple Range Test. Statements of statistical significance are based on P<0.05. These analyses were accomplished by using a statistical analysis system configured for computer (SPSS, Relase 10.0, SPSS. Inc).

RESULTS

The mean STT-1 and STT-2 values in Turkish Hunting Dogs were 17.1 ±3.7 mm per minute and 7.7 ±2.8 mm per minute, respectively (Table 2). There were significant daily and weekly fluctuations for STT-1 and STT-2 values (P<0.01, P<0.05, respectively (Table 2)).

The 'Young' age group contained five Turkish Hunting Dogs (3 females and 2 males), ranging from 6 months to 2 years in age (mean 12.4 ±5.4 months). The mean body weight was 17.1 ±2.4 kg (ranging from 14 to 20.5 kg). The mean values for STT-1 and STT-2 were 16.2±3.3 mm/min and 7.7 ±2.2 mm/min, respectively (Table 3).

The 'Middle' age group contained five Turkish Hunting Dogs (2 females and 3 males), ranging from 2 to 5 years in age (mean 3.8 ±0.8 years). The mean body weight was 21.7 ±1.5 kg (ranging from 20 to 24 kg). The mean values for STT-1 and STT-2 were 16.6± 3.5 mm/min and 7.1 ±2.9 mm/min, respectively (Table 3).

The 'Old' age group contained five Turkish Hunting Dogs (3 females, 2 males), ranging from 6 to 9 years in age (mean 6.8 ±0.9 years). The mean body weight was 24.2 ±1.6 kg (ranging from 22 to 27 kg). The mean values for STT-1 and STT-2 were 18.3 ±4.3 mm/min and 8.5 ±3.3 mm/min, respectively (Table 3).

The mean values for STT-1 and STT-2 in the female dogs were 17.9 ±3.4 mm/min and 16.3 ±3.7 mm/min, respectively. For the male dogs, these values were 8.3 ±2.4 mm/min and 7.2 ±3.0 mm/min (Table 3). Both the STT-1 and the STT-2 values were significantly different in females and males (P<0.05). On the other hand, there were no statistically significant differences in the STT-1 or STT-2 values related either to body weight or to age group (P>0.05).

The mean morning and afternoon values were 16.4 ±3.6 mm/min and 17.8 ±4.2 mm/min for STT-1, and 7.0 ±2.9 mm/min and 8.3 ±3.3 mm/min for STT-2, (Table 3). The morning and afternoon values were significantly different both for STT-1 and STT-2 ($P<0.05$).

DISCUSSION

The Schirmer tear test (STT-1) and modified Schirmer tear test (STT-2) with topical anesthesia for measuring tear production in animals have been described by a number of authors (Gelatt *et al.*, 1975; Rubin *et al.*, 1965; Berger and King, 1998; Saito and Kotani, 2001). However, only STT-1 is routinely used in clinics because of its relatively quick and easy procedure (Rubin *et al.*, 1965; Hakanson and Arnesson, 1997; Hamor *et al.*, 2000). According to previous reports, the normal values for STT-1 and STT-2 in healthy dogs range from 15.8 ±1.8 to 22.9 ±4.1 mm per minute and from 3.6 ±2.8 to 11.6 ±6.1 mm per minute, respectively (Rubin *et al.*, 1965; Gelatt *et al.*, 1975; Izci *et al.*, 1995; Hamor *et al.*, 2000; Alkan *et al.*, 2004). In this study, it was documented that the mean values for STT-1 and STT-2 in the Turkish Hunting Dogs were 17.1 ±3.7 mm/min and 7.7 ±2.8 mm/min, respectively (Table 2). The study has shown that STT-1 and STT-2 values in apparently healthy Turkish Hunting Dogs are consistent with the values reported in other dogs (Rubin *et al.*, 1965; Gelatt *et al.*, 1975; Izci *et al.*, 1995; Hamor *et al.*, 2000; Alkan *et al.*, 2004) (Table 2). Moreover, in the present study, we observed that the mean STT-2 values were approximately 45.1 % of the STT-1 values. We believe that this decrease is due to the drug's anesthetic effect on the cornea, which results in blockage of the afferent pathway of the reflex, and the prevention of reflectory secretion by the lacrimal and nictitans glands. This is in accordance with earlier reports that showed the mean STT-2 value to be approximately 30.5% to 58.5% of the STT-1 values (Aguirre *et al.*, 1995; Berger and King, 1998; Saito and Kotani, 2001; Alkan *et al.*, 2004).

The STT-1 and STT-2 are the most popular tests for the examination of tear production in dogs of different breeds (Wyman *et al.*, 1995; Izci *et al.*, 1995; Hakanson and Arnesson, 1997; Hamor *et al.*, 2000; Saito and Kotani, 2001; Alkan *et al.*, 2004). However, it has been documented that head shape, body weight and age have no effect on the STT values in dogs (Rubin *et al.*, 1965; Wyman *et al.*, 1995; Hamor *et al.*, 2000; Alkan *et al.*, 2004). On the other hand, according to Berger and King (1998), the values of the STT-1 and the STT-2 may be positively correlated with the dog's weight. However, in the present study the related values were found to be a little higher for the group with old age and higher body weight than for the other groups. One would expect that head shape would influence the STT results because the corneal sensitivity is related to skull type (Hamor *et al.*, 2000). In the light of these data, smaller dogs may be more likely to develop KCS as they age. Although some authors (Rubin *et al.*, 1965; Berger and King, 1998; Hamor *et al.*, 2000) have stated that gender has no effect on the STT-1 and STT-2 values, we found these values to be higher in females than in males, as reported in earlier communications (Alkan*et al.*, 2004; Hakanson and Arnesson, 1997; Wyman *et al.*, 1995). The differences between females and males can be attributed to the female sex hormones, which have a stimulating effect on lacrimation. Deficiency in female sex hormones can contribute to the dysfunction of tear production glands and can lead to KCS.

It has been suggested that the STT-1 and STT-2 values may be related to the dog's breed (Hakanson and Arnesson, 1997; Hamor *et al.*, 2000; Saito and Kotani, 2001; Alkan *et al.*, 2004). However, another report denied such a variation (Rubin *et al.*, 1965). Previously reported STT-1 values in various breeds include 17.4 ±5.6 mm/min in the Poodle (Rubin *et al.*, 1965), 18.9 ±2.6 mm/min in the Beagle (Saito and Kotani, 2001), 22.9 ±4.1 mm/min in the Labrador Retriever, 20.7 ±3.2 mm/min in the English Springer Spaniel, 21.8 ±3.7 mm/min in the Golden Retriever, 15.8 ±1.8 mm/min in the Shetland Sheepdog (Hamor *et al.*, 2000), and 21.1 ±3.4 in the Turkish Shepherd Dogs (Alkan *et al.*, 2004). In our study, it was determined that the mean STT-1 values were 17.1 ±3.7 mm/min in Turkish Hunting Dogs (Table 2). In addition, the mean STT-1 values in Turkish Hunting Dogs were found to be consistent with most other breeds. Previously reported STT-2 values in various breeds include 9.5 ±4.5 mm/min in the Beagle (Saito and Kotani, 2001), 9.6 ±3.8 mm/min in the Labrador Retriever, 5.4 ±3.4 mm/min in the English Springer Spaniel, 8.8 ±3.1 mm/min in the Golden Retriever, 3.6 ±2.8 mm/min in the Shetland Sheepdog (Hamor *et al.*, 2000), and 9.8 ± 2.7 mm/min in the Turkish Shepherd Dog (Alkan *et al.*, 2004). In the present study, the STT-2 value for Turkish Hunting Dogs (7.7 ±2.8 mm/min) was very similar to the values reported for all other breeds (Table 2). The breed differences

should be taken into consideration as a contributing factor during the clinical examinations of dogs, whether healthy or affected by KCS.

It was previously reported that there are daily, weekly and diurnal fluctuations in STT-1 and STT-2 values in healthy dogs (Izci *et al.*, 1995; Smith *et al.*, 1995; Hakanson and Arnesson, 1997; Berger and King, 1998; Alkan *et al.*, 2004). The results of the present study indicate that the dogs have typical fluctuations in daily, weekly and daytime tear production ($P<0.01$, $P<0.05$, $P<0.05$, respectively). The daytime changes are lowest at midday and highest in the late afternoon/early evening in diurnal variation, which is in agreement with Smith *et al.* (1995) and Alkan *et al.*, (2004). It should be noted that repetitive testing may induce changes in both STT-1 and STT-2 values due to irritation, stress, or physical condition.

Our study has shown that STT-1 and STT-2 values in Turkish Hunting Dogs are normally distributed and are significantly affected by breed, sex and/or measurement sequences. Therefore, we propose that data on age, body weight, sex, head type, breed and ecological conditions should be taken into consideration together with all STT measurements so that tear production can be accurately determined. We think that further studies are needed to properly appreciate the differences among various canine breeds in order to avoid the misdiagnosis of KCS.

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