



## NOTE

Internal Medicine

# The effect of environmental and biological factors on STT I and normal total tear protein concentration in Japanese black calves

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**ABSTRACT.** To identify reference values for the Schirmer tear test I (STT I) in Japanese black cattle, investigate the effects of variables (sex, age, time of day, and environment in the barn including environmental temperature, humidity, illuminance, and ammonia concentration) on tear production, and determine total tear protein concentration. One-hundred-and-thirty-seven Japanese black cattle (67 females and 70 males, age 3–90 days) were evaluated. The mean STT I values was  $18.9 \pm 2.9$  mm/min ( $n=263$ ) and significant effects were age and ammonia concentration in the barn. Mean total tear protein concentration was  $1.18 \pm 0.30$  mg/ml in healthy cattle older than 15 days ( $n=38$ ). It was suggested that age and ammonia concentration are related to fluctuation of tear volume.

**KEY WORDS:** Japanese black calf, lacrimal fluid, ruminants, Schirmer tear test, total tear protein

The precocular tear film can be divided into three layers. The outer layer is a very thin, oily layer, consisting of waxy and cholesterol esters [4, 10]. The middle layer is the thickest and performs the primary functions of the tear film; this layer is composed of approximately 98% water and 2% solids, made up predominantly of proteins [6]. The deep layer is composed of mucins produced by the apocrine conjunctival goblet cells. The Schirmer tear test (STT) is used to evaluate the aqueous tear layer, which is critical for corneal and conjunctival health, is evaluated via STT. The STT has been widely used in both human and veterinary ophthalmology as a basic assessment of tear production. The STT I measures basal and reflex tearing. The STT quantifies production of the aqueous component of the tear film [10]. Normal STT values have been established in a number of domestic and wild animals [1, 3, 7, 11, 13, 17, 18].

Previously published reports have documented STT values in clinically normal adult cattle [19, 20] and Holstein calves [16]. However, there are no reports of ophthalmic findings in Japanese black cattle. In recent years, there is a surge in the popularity of Japanese black cattle. Determining the baseline value of total tear protein concentration is essential for future investigations into its influence on the ocular surface health and comfort Japanese black cattle.

The purpose of the present study was to clarify the normal reference range of the STT I value and total tear protein concentration in healthy Japanese black cattle. The effects of age, sex, environmental temperature, humidity, illuminance, and ammonia concentration in the barn, and time of day on tear production were also investigated.

The present study was approved by the ethics committee of our institution. The study population was comprised of 137 Japanese black cattle (70 males and 67 females, age range 3 to 90 days). All animals were examined in their normal environment, and were considered clinically normal based on a brief physical examination. Each calf was either a livestock calf that had been isolated from the dam and was being fed milk substitute and starter, or a calf living in the same space as the dam and suckling plus being fed starter. Each livestock calf isolated from the dam lived with the dam and ingested colostrum until it was 7 days old, and was then individually housed indoors. All animals had no signs of disease of the external ocular structures, and had no history of ocular disease. Assuming that the calf's dairy gain is about 0.7 kg, it increases by 10 kg after 15 days. As a group setting for comparison with calves whose body weight was increased by 10 kg, measurements were made at every 15 days of age.

The same veterinarian performed all the testing. Rectal temperature, heart rate, and respiratory rate were measured. A multi-environment measuring instrument (LM-8000, MOTHERTOOL Co., Ltd., Nagano, Japan) was used to measure the environmental

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temperature, humidity, and illuminance around the animal's body at a position approximately 15 cm from the body surface around the animal's eyes. The ammonia concentration in the barn was measured using a gas detector (GV-100S, GASTEC Corp., Ayase, Japan) positioned approximately 15 cm from the body surface around the animal's eyes. An ammonia concentration of 2 ppm or more was considered to produce an offensive odor, in accordance with the Offensive Odor Control Law.

The STT I was performed to measure aqueous tear production. Each STT strip (Schirmer Tear Test Standardized Sterile Strips, Distributed by Intervet Inc. Merck Animal Health, Summit, NJ, U.S.A.) was placed into the lateral conjunctival fornix of the lower eyelid for 1 min, at a position approximately one-third of the distance from the lateral canthus to the medial canthus. After collecting the STT strip, it was measured immediately in mm/min. Tests were always performed on the left eye first, followed by the right eye. The STT I was performed at random times between 10:00 to 19:00. The time when the STT I was performed was recorded.

After measuring STT I of both eyes, tear samples from both eyes were collected by placing 10  $\mu$ l glass microcapillary tubes (Drummond MICROCAPS, Drummond Scientific Co., Broomall, PA, U.S.A.) in the lower conjunctival fornix. In most cattle, tears were obtained by capillary action. Tears were always collected from the left eye first, followed by the right eye. Care was taken to cause as little conjunctival trauma as possible during tear collection. The tear sample from each eye was placed in a separate tube and stored in liquid nitrogen until used. The concentration of protein in the sample was measured using the Bradford assay (pierce 660 nm Protein Assay, Thermo Fisher Scientific, Waltham, MA, U.S.A.) with a spectrophotometer wavelength of 660 nm.

All data are presented as the mean  $\pm$  standard deviation. The paired Student's *t*-test was used to evaluate differences in STT I values between the right and left eyes and between females and males. The differences in STT I values in accordance with age were analyzed via one-way analysis of variance followed by *post hoc* with Tukey-Kramer testing. The correlations between STT I values and environmental temperature, humidity, illuminance, and ammonia concentration, and time of day were determined by Spearman's correlation coefficient by rank test. The paired Student's *t*-test was used to evaluate differences in total tear protein concentration between healthy cattle older than 15 days and those younger than 15 days. A value of  $P < 0.05$  was considered as statistically significant. Statistical analyses were performed using Excel 2010 with Statcel 3 add-in software.

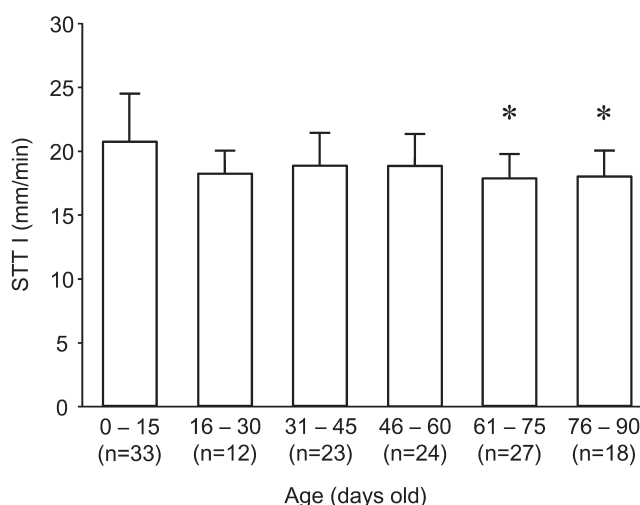
The mean STT I value was  $18.9 \pm 2.9$  mm/min when measurements from both eyes were included (263 eyes from 137 Japanese black cattle). The mean STT I values for the right and left eyes were  $18.7 \pm 3.2$  (n=130) and  $19.0 \pm 3.0$  (n=133) mm/min, respectively. The mean STT I values for males and females were  $18.6 \pm 2.6$  (n=70) and  $19.1 \pm 3.1$  (n=67) mm/min, respectively. There were no significant differences in the STT I values of the right and left eyes, or between males and females. The STT I value ( $20.6 \pm 3.8$  mm/min) of cattle younger than 15 days (n=33) was significantly increased compared with those older than 60 days (n=45) ( $17.7 \pm 1.9$  mm/min) ( $P < 0.05$ , Fig. 1). Therefore, the statistical analyses of environmental variables were limited to cattle older than 15 days. There were no significant correlations between STT I values and the environmental temperature, humidity, and illuminance, or the time of day (Fig. 2). However, the STT I value was significantly positively correlated with the ammonia concentration in the barn ( $P < 0.05$ ) (Fig. 3).

The total tear protein concentration was  $1.18 \pm 0.30$  mg/ml in healthy cattle older than 15 days (n=38). In calves younger than 15 days, the total tear protein concentration was  $0.95 \pm 0.36$  mg/ml (n=9). There was no significant difference in the total tear protein concentration between healthy cattle older than 15 days and those younger than 15 days ( $P < 0.05$ ).

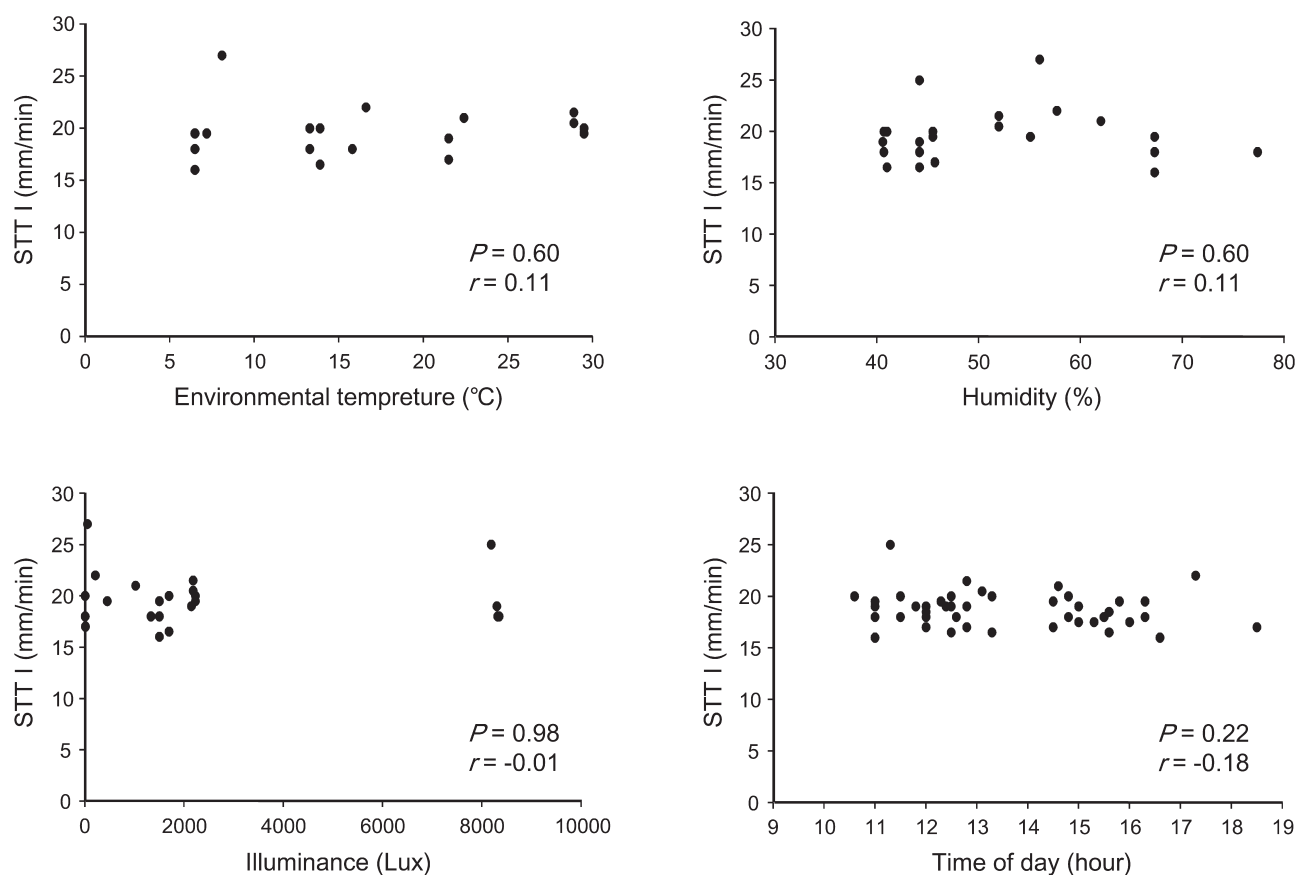
The middle aqueous tear layer of the preocular tear film is evaluated clinically via the STT [12]. The STT I quantifies the production of the aqueous component of the tear film [6].

The present study provides preliminary data for the reference ranges of tear production in clinically normal Japanese black cattle calves. The mean STT I value for all Japanese black cattle evaluated was  $18.9 \pm 2.9$  mm/min, which is comparable to the reported STT I values for domestic ruminants, such as sheep and goats [7, 14, 16]. The respective mean STT I values in normal sheep and goats are reportedly  $18.5 \pm 2.5$  mm/min (range 13.5–22 mm/min) and  $15.8 \pm 5.7$  mm/min (range 10–30 mm/min) [3, 7, 20]. The previous studies reported that mean STT I values in Holstein calves aged 9.3–13.3 weeks was  $20.4 \pm 5.0$  mm/min [16], which was lower than those in adult cattle were as  $34.15 \pm 20.47$  mm/min [20] and  $24.18 \pm 6.5$  mm/min [19]. The present results for STT I values in Japanese black cattle calves was similar to that in Holstein calves.

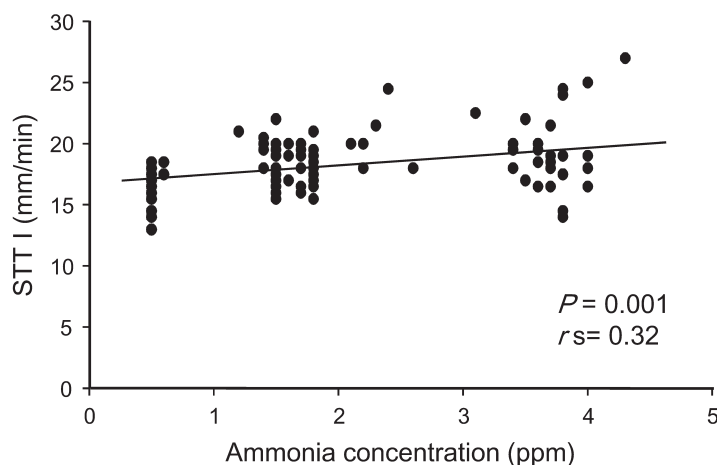
The present study demonstrated that the STT I value in Japanese black cattle was significantly affected by age and the ammonia concentration in the barn. In dogs, variations in STT I values in accordance with age, sex, and weight have been studied previously, with conflicting results. Hartley *et al.* demonstrated that STT I values decrease with age in normal dogs [9]. Broadwater *et al.* reported that STT I values are significantly affected by age, weight, and sex in juvenile



**Fig. 1.** Schirmer tear test (STT) I values in accordance with age in Japanese black cattle. The STT I value of cattle younger than 15 days was significantly greater than that of cattle older than 60 days. \* $P < 0.05$  compared with cattle aged 0 to 15 days.



**Fig. 2.** Correlations between the Schirmer tear test (STT) I value and environmental temperature, humidity, and illuminance, and time of day. There were no significant correlations between the STT I value and any of the assessed variables.



**Fig. 3.** Correlation between the Schirmer tear test (STT) I value and the ammonia concentration in the barn. The STT I value was significantly positively correlated with the ammonia concentration in the barn ( $P=0.001$ ,  $r_s=0.32$ ).

dogs, and that STT I values appear to increase to normal adult values by 9–10 weeks days of age [2]. However, although this previous study included a wide age range of juvenile dogs, it was unable to determine whether the tear production increases with age, and to what extent [2].

Calves that are only a few days old must adapt to harsher environments than the environment in the womb. The increased amount of tear production in young calves (younger than 15 days) compared with cattle older than 60 days in the present study suggests the important role of tear production in ocular surface maintenance in harsh conditions. In addition, the precorneal tear

film contains both nonspecific and specific antimicrobial substances. The specific antimicrobial substances are produced by the corneal and conjunctival epithelial cells, and aid in the defense against many types of microbial infections [6]. In a barn with a high ammonia concentration, the mucosa of the calf's eyes is continually stimulated. Hence, the increased tear production in calves may be due to the need to accommodate to the relatively harsh environment. In the present study, it is suggested that a high value of tear volume was observed in a barn with an ammonia concentration of higher than 2 ppm.

The present study demonstrated that tear production in Japanese black cattle calves was not significantly affected by environmental temperature, humidity, and illuminance, and time of day. The environment inside the barn seems to be maintained at a comfortable level throughout the year. Even when the outside temperature is below freezing in the winter, there are no substantial changes in the barn environment as the barn contains measures that minimize the cold (such as doors and heaters). In contrast with the present findings, Piccione *et al.* reported that rhythmicity significantly affects horse tear production [15]; however, Beech *et al.* found no daily rhythmicity in horse tear production [1]. The STT I values in horses reportedly vary depending on the time of day, with the lowest values observed at 8:00, followed by a gradual increase until the acrophase (between 16:00 and 17:00), and a gradual decrease until 8:00 on the second day [15]. However, in the present study, the STT I values measurements were taken at random times between 10:00 and 19:00, and the light timers were not set to maintain 12 hr of light and 12 hr of darkness each day. Hence, it remains unclear whether the STT I values were affected by circadian rhythms in Japanese black cattle.

The present study is the first to report the normal total tear protein concentration in Japanese black cattle, although data are available on the total tear protein concentration in other adult cattle breeds. The total tear protein concentration of cattle has previously been reported as  $5.8 \pm 2.2$  mg/ml [5] and  $4.4 \pm 1.9$  mg/ml [8]. Our results are lower than these previous results.

In conclusion, the present study provides novel data for the normal reference ranges of STT I values in healthy Japanese black cattle. We revealed that STT I values vary with days from birth and ammonia concentration in Japanese black cattle. In measuring STT I for further studies, there is need to account for days from birth and ammonia concentration. Also, further studies are required to examine the mechanism why the STT I value of cattle younger than 15 days are increased compared with those older than 15 days, and to measure the total tear protein concentration in Japanese black cattle.

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