Reference values and repeatability of the Schirmer tear tests I and II in domesticated, clinically normal dromedary camels (Camelus dromedarius)

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Abstract

Objective To determine the normal values and repeatability for Schirmer tear test A. M. Badawy (STT) in clinically normal dromedary camels and to analyze the influence of the age Tel.: +20 01118859378 and gender on these values. Fax: +20 013 3461411 Animals studied Thirty clinically normal dromedary camels of different ages (calves, adelbadawybadawy@yahoo.com; immature, and mature). adel.badawy@fvtm.bu.edu.eg

Procedures Schirmer tear tests I and II were performed using commercial STT strips. Three measurements were obtained from each eye over three consecutive weeks, and the variance of these measurements was determined.

Results Mean values and coefficient of variation of STT I and STT II for the right and left eyes varied significantly among camel groups (P < 0.05). For STT I, the most frequently recorded values were >14-18, > 22-26, and >30-34 mm/min in calves, immature camels, and mature camels, respectively. For STT II, however, the most frequently recorded values were 7-14, >10-18, and >26-30 mm/min, respectively. The interassay coefficients of variation were 1.7-14.4% and were significantly lower in mature camels than in calves and immature camels (P < 0.05). Age was positively correlated with STT I (r = 0.81) and STT II values (r = 0.88). No significant variations were found between genders.

Conclusions This preliminary study reports STT I and II values and repeatability in normal dromedary camels. This information may assist veterinary practitioners in complete ophthalmic examinations and in accurate diagnosis of ocular surface diseases affecting the tear film in this species.

Key Words: camel, camelids, dromedary, Schirmer tear test, Schirmer tear test I, Schirmer tear test II

INTRODUCTION

Camel (Camelus dromedarius) has a unique tear productiondrainage system that differs greatly from that of the other domestic animals. For this reason, studying tear production in the camel would add important information to the field of comparative ophthalmology. The lacrimal gland in the camel is small when compared with the animal's size, and its excretory ducts (afferent lacrimal ducts) are few in number (3-4) with wide openings.^{1,2} In contrast to other domestic animals, the lacrimal puncta are very small or absent in camels.¹⁻³ These unusual features motivated the authors to test tear production in the dromedary camel and compare the results with those recorded in other animals. Because there is very little work published on ocular diseases and ophthalmic diagnostic tests in the dromedary camel information on normal ranges, repeatability of the STT is important for this species.^{4–6}

Ocular diagnostic tests commonly used in veterinary medicine include the Schirmer tear test (STT), tonometry, and fluorescein staining. Schirmer tear test is an important component of a complete ophthalmic examination in both human and veterinary patients, as it assesses tear production which is critical for corneal and conjunctival health.

Evaluation of the amount of tears produced by animals is an important diagnostic tool, particularly if a deficiency is suspected.⁷ Two types of STT have been described, STT I and STT II.⁸ STT I measures basal and reflex tear production, while STT II evaluates basal tear production after the application of a topical anesthetic.^{9,10}

Conjunctivitis, superficial keratitis, corneal ulceration, and impaired healing of corneal ulcerations are common ocular diseases in dromedary camels. These conditions are frequently associated with inadequate tear production.¹¹ Knowledge of STT values in clinically normal dromedary camels is essential for the assessment of tear production and would be a useful indicator in the diagnosis and monitoring of corneal and conjunctival disease. Normal STT physiological parameters have been established in a number of domestic, wild, and exotic animal species.7,9-21 To the authors' knowledge, there are no normal values for STT I and II in dromedary camels in the published veterinary literature. Therefore, the aim of this study was to determine the normal values and repeatability for STT I and STT II values in clinically normal dromedary camels and to analyze the influence of age and gender on these values.

MATERIALS AND METHODS

Animals

Thirty clinically normal dromedary camels (16 male and 14 female) were used in this study. All animals were part of the same herd in a private farm in Qaliubyia, Egypt and were housed together in a fenced outdoor paddock with some form of shelter. These camels were raised for milk, meat, and transport. The camels were divided into three groups (10 camels/group, five males and five females) based on age as follows: the calves group, <6 months of age, the immature camels group, >6 months but <5 years of age and the mature camels group, 5–10 years of age.

The camels were allowed *ad libitum* access to food and water until the beginning of each procedure. All animals underwent a complete ophthalmic examination, including fluorescein staining, slit-lamp biomicroscopy, and indirect ophthalmoscopy 24–48 h before and after the procedures. Camels were only included in the study if they had no previous history of ocular or systemic disease and exhibited no signs of adnexal or ocular abnormalities during the ophthalmic examination.

Study approval

The study protocol was approved by the Animal Care Committee of the Kafer-Elsheikh and Benha Universities, in accordance with Egyptian ethical codes for studies on experimental animals. Additionally, owner consent was also obtained for the use of the camels prior to the study.

STT I and II measurements

All testing was performed indoors in a familiar, quiet environment, and all camels were physically restrained by halters in a sitting position by the same individuals. In all three groups, all measurements were recorded by the same examiner. All measurements were obtained between 8:00 a.m. and 11:00 a.m. on the same day of the week for three consecutive weeks during the month of April. The order of eyes examined (right vs. left) in each camel was randomized using a simple randomization method. Neither chemical sedation nor local nerve blocks were employed. All STT strips used came from a single batch, with the same lot number.

In all camels, STT I was performed in both eyes by placing a standard 5×35 mm (i.e., maximum reading 35 mm), sterile STT strip (Schirmer-Tränentest, Essex Pharma GmbH, Munich, Germany), in the lateral aspect of the lower conjunctival fornix for 1 min. The strip was then removed, and the amount of tear production was measured immediately and recorded in mm/min. For STT II, the ocular surface was anesthetized via the application of two drops of 0.5% proparacaine ophthalmic solution into each conjunctival sac (proparacaine hydrochloride 0.5% ophthalmic solution; Alcaine, Alcon Laboratories, Inc., Fort Worth, Texas, USA). One minute after the application of proparacaine solution, the conjunctival fornices were gently dried using a sterile swab (Sterile cotton swabs, F.L. Medical s.r.l., Torreglia (Padova)-Italy). STT II was then performed as for the STT I. Camel tolerance of the procedures and the results of ophthalmic examinations before and after the STT I and II were recorded.

The measurements were repeated three times in each camel, 1 week apart, by the same investigator under identical conditions to determine interassay coefficients of variation (CVs). Thus, by the end of the study, there were three STT I and three STT II measurements for each eye from each camel.

Data analysis

All statistical procedures were performed with commercially available software (GraphPad Prism for Windows version 5.0; GraphPad Software Inc., CA, USA). The distributions of the STT I and STT II data were assessed via the Kolmogorov–Smirnov normality test, and the data were determined to be normally distributed. The frequency distribution and the summary statistics (mean \pm SD, 95% CI, median, range, and 10th, 25th, 75th, and 90th percentiles) of the STT I and II values for the right and left eyes of each group of camels were calculated.

The repeatability of the STT measurements was evaluated by calculating the interassay CVs. For each of the 3 days that data were collected, the interassay CV was calculated by dividing the SD of the measurement by the mean of the same measurement. A one-way ANOVA with *post hoc* Turkey's multiple comparisons test was used to compare the means of STT measurements and interassay CVs among the different groups of camels. Paired t-tests were used to compare mean STT measurements and interassay CVs between right and left eyes. Unpaired

Category	Calves		Immature		Mature	
	Right	Left	Right	Left	Right	Left
STT I						
11-14	3 (10.0)	7 (23.3)	0 (0)	0 (0)	0 (0)	0 (0)
>14-18	17 (56.7)	14 (46.7)	0 (0)	0 (0)	0 (0)	0 (0)
>18-22	6 (20.0)	8 (26.7)	6 (20.0)	5 (16.7)	0 (0)	0 (0)
>22-26	4 (13.3)	1 (3.3)	14 (46.7)	19 (63.3)	3 (10.0)	2 (6.7)
>26-30	0 (0)	0 (0)	10 (33.3)	6 (20.0)	11 (36.7)	10 (33.3)
>30-34	0 (0)	0 (0)	0 (0)	0 (0)	16 (53.3)	18 (60.0)
STT II						
7-10	13 (43.3)	15 (50.0)	1 (3.3)	1 (3.3)	0 (0)	1 (3.3)
>10-14	15 (50.0)	13 (43.3)	11 (36.7)	11 (36.7)	0 (0)	0 (0)
>14-18	2 (6.7)	2 (6.7)	10 (33.3)	14 (46.7)	0 (0)	0 (0)
>18-22	0 (0)	0 (0)	8 (26.7)	3 (10.0)	1 (3.3)	2 (6.7)
>22-26	0 (0)	0 (0)	0 (0)	1 (3.3)	9 (30.0)	9 (30.0)
>26-30	0 (0)	0 (0)	0 (0)	0 (0)	20 (66.7)	18 (60.0)

Table 1. Frequency distribution (% of study population) of STT I and STT II measurements (mm/min) in 30 clinically normal dromedary camels (*Camelus dromedarius*)

STT = Schirmer tear test. Values represent the number (%) of measurements in that particular STT I or STT II category for the given eye. The STT I and STT II measurements were 0–20 mm/min. The calves were aged <6 months, the immature camels were aged >6 months but < >5 years. For each camel, three measurements were obtained for each eye, at 1-week intervals. Thus, there were 30 measurements for each eye for each group.

Table 2. Summary of STT I and STT II measurements (mm/min) in 30 clinically normal dromedary camels (Camelus dromedarius)

	Calves		Immature		Mature	
Variable	Right	Left	Right	Left	Right	Left
STT I						
Mean \pm SD	$18.0 \pm 3.2^{\mathrm{a}}$	16.7 ± 2.9^{a}	$24.9\pm2.9^{\rm b}$	$25.0\pm2.8^{\rm b}$	$30.4\pm2.8^{\rm c}$	$30.7 \pm 2.3^{\circ}$
95% CI of mean	16.8-19.2	15.6-17.8	23.9-26.1	23.9-26.1	29.4-31.5	29.8-31.6
Minimum–Maximum	13-25	11-23	20-30	20-30	24-34	24-34
STT II						
Mean \pm SD	11.2 ± 2.5^{a}	10.6 ± 2.5^{a}	$15.9\pm3.2^{\rm b}$	$15.6\pm3.07^{ m b}$	$27.4 \pm 2.3^{\circ}$	$26.8 \pm 1.9^{\rm c}$
95% CI of mean	10.2 - 12.1	9.7-11.5	14.7 - 17.1	14.5 - 16.8	26.6-28.3	26.1-27.6
Minimum–Maximum	7.0–18.0	7.0–16.0	10.0-21.0	10.0-23.0	22.0-30.0	21.0-30

^{a,b,c}Indicates significant difference within row at P < 0.05.

t-tests were used to compare STT I and II values and to compare data from males and females. The STT measurements obtained for each eye of each camel on the first data collection day were used to assess the correlation between age and STT measurements, via Pearson's correlation coefficient. For all analyses, *P* values <0.05 were considered significant.

RESULTS

All camels were considered healthy on the basis of a physical examination and complete blood count. Their ages were as follows: the calves group, <6 months of age with a mean \pm SD age of 2.6 \pm 1.9 months, the immature camels group, >6 months but <5 years of age with mean \pm SD age of 2.7 \pm 1.1 years, and the mature camels group, 5–10 years of age with mean \pm SD age of 7.8 \pm 1.9 years.

Both STT I and STT II were easy to perform and well tolerated in all camels, without the need for sedation or local nerve blocks, and neither test appeared to cause ocular irritation or undue stress to the animals. The results of the pre- and post-STT I, STT II ophthalmic examinations were normal.

The frequency distributions of STT I and II measurements in calves, immature, camels, and mature camels are summarized in Table 1. For STT I, the most frequently recorded measurements were >14–18, >22–26, and >30–34 mm/min in calves, immature, camels, and mature camels, respectively. For STT II, the most frequently recorded measurements were 7–14, >10–18, and >26–30 mm/min, respectively.

All the STT I and II data are expressed as mm/min. There was significant variation in the STT measurements among the different groups (Table 2). For the calves group, the mean \pm SD STT I values for the right and left eyes were 18.03 \pm 3.16 and 16.7 \pm 2.99 mm/min, respectively. The mean \pm SD STT II values were 11.17 \pm 2.48 mm/ min in the right eyes and 10.6 \pm 2.5 mm/min in the left eyes. For the immature camel group, the mean \pm SD STT I values for the right and left eyes were 24.9 \pm 2.9 and 25.0 \pm 2.8 mm/min, respectively. The mean \pm SD STT II

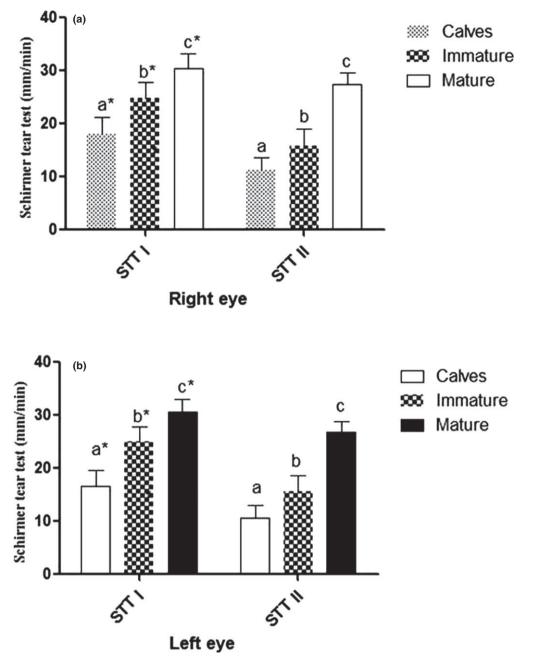


Figure 1. Mean +/- SD STT I and STT II results (mm/min) in right (a) and left (b) eyes of dromedary camels (*Camelus dromedarius*). *Indicates that STT I differs significantly from STT II for the corresponding age. a,b,c indicate significant variation among ages.

values were 15.9 ± 3.2 mm/min in the right eyes and 15.6 ± 3.07 mm/min in the left eyes. For the mature camel group, the mean \pm SD STT I values for the right and left eyes were 30.4 ± 2.8 and 30.7 ± 2.3 mm/min, respectively. The mean \pm SD STT II values were 27.4 ± 2.3 mm/min in the right eyes and 26.8 ± 1.9 mm/min in the left eyes.

Schirmer tear test II values were significantly lower than STT I values in both the right and left eyes in all camel groups (P < 0.05) (Fig. 1). Variations in STT I and II values between the right and left eyes and between males and

females in the different groups were not significant. The interassay CVs for the right and left eyes in each group are summarized in Table 3. The interassay CVs were significantly lower in mature camels than in calves and immature camels (P < 0.05). However, the difference in variation between calves and immature camels was not significant. Age was positively correlated with STT I (r = 0.81) and STT II (r = 0.88) measurements. A 4-month-old camel exhibited the lowest STTI and STT II values (13 and 11 mm/min for the right and left eyes respectively), and a 10-year-old camel exhibited the

	Calves		Immature		Mature	
Variable	Right	Left	Right	Left	Right	Left
STT I						
Mean \pm SD	8.3 ± 3.3^{a}	8.4 ± 1.7^{a}	10.3 ± 2.8^{a}	9.9 ± 3.2^{a}	$6.7~\pm~3.1^{ m b}$	4.2 ± 1.6^{b}
95% CI of mean	5.8-10.6	7.2-9.6	8.4-12.3	7.6-12.2	4.4-8.9	3.0-5.3
Minimum–Maximum	3.5-15.80	6.7-11.1	4.8-14.4	4.6-14.4	3.0-14.0	1.7 - 6.5
STT II						
Mean \pm SD	12.6 ± 5.8^{a}	11.2 ± 4.0^{a}	14.2 ± 3.1^{a}	11.5 ± 6.3^{a}	$5.5~\pm~3.1^{ m b}$	4.1 ± 1.7^{b}
95% CI of mean	8.4-16.8	8.3-14.1	11.9-16.4	6.9-16.0	3.3-7.7	2.9-5.3
Minimum-Maximum	5.1-24.5	4.6-20.2	9.9-19.5	3.3-23.5	1.9-10.2	2.1-7.4

Table 3. Interassay coefficients of variation (%) of STT I and STT II measurements in 30 clinically normal dromedary camels (*Camelus dromedarius*) assessed once weekly for 3 weeks.

^{a,b,c}Indicates significant difference within row at P < 0.05.

highest STT I and II measurements (34 mm/min for both the right and left eyes).

DISCUSSION

The results of the present study provide preliminary normal ranges for tear production in healthy dromedary camels. Camels reach puberty at approximately 5 years of age;²² therefore, in the present study, camels aged \geq 5 years were categorized as mature. It should be noted that there were significant differences in STT I and STT II values among the three groups of camels evaluated in the present study. The STT I and II values of mature camels were significantly higher than those of both juvenile and immature camels. Similar observations have previously been reported in humans,²³ horses,²⁴ and pigs.²¹

The authors believe that ocular surface disease is often overlooked or misdiagnosed in camels, as complete ophthalmic examination of these animals is generally not performed in the field due to a lack of suitable specialized facilities and equipment. Therefore, the aims of the study reported herein were to determine the STT I and II values of clinically normal dromedary camels of various ages and assess the repeatability of STT I and II measurements in camels.

The camel is a well-known desert animal (also known as the 'ship of the desert') and is well adapted to high temperature, scorching sun rays, and a sandy, windy, dry climate. The camel's eyes must be provided with a continuous lacrimal secretion to moisten and wash the ocular surface free of irritants, foreign particles, and fumes. The lacrimal system provides a passage for drainage from the eye to the nasal cavity. The nasolacrimal apparatus of the camel consists of a simple lacrimal sac and paired canaliculi with small dorsal and ventral puncta. Previous studies have reported that the puncta lacrimalis in dromedary camels are remarkably small or absent.^{1–3} It seems that the very small-sized puncta lacrimalis causes excess tears to escape by flowing over the lower eyelid.²⁵

The mean STT I for camel calves was similar to that reported in several other domestic ruminants and

camelids, including bovine calves, adult llamas, pygmy goats, and Sanjabi sheep.^{8,12,26,27} Similarly, the mean STT I for both immature and mature camels was comparable to that reported in adult cattle and horses. It is interesting to note that the mean STT values obtained in this investigation in immature camels and mature camels are dissimilar to those reported for the closely related llama. The reported means of STT I and II values in normal llama are 17.3 \pm 1.1 mm/min (15–19 mm/min) and 15.4 \pm 1.7 mm/min (12.5-17.5 mm/min), respectively.²⁷ We speculate that differences in tear production might be due to differences between species in the size of the conjunctival sac, or the available tear reservoir on the surface of the eve. Additionally, the llama is a smaller animal, with a body mass smaller than that of the camel, which may contribute to the large differences in STT values between the two species.

In the current study, there were no significant differences between males and females with regard to STT I or STT II values. These results are similar to those previously reported in several species, including the pygmy goat, Nubian ibex, Burcell's zebra, chinchilla, koala, pig, llama, and calf.^{8,14,21,26-31}

In this study, in all groups, the mean STT II value was significantly lower than the corresponding mean STT I value. These observations are similar to those previously reported in dogs, cats, and pigs.^{21,32–34} However, some authors have observed no significant differences between STT I and II values in horses and guinea pigs,^{9,35} whereas others have observed a minimal difference in horses.³⁶

Multiple factors are known to significantly influence tear production, so we attempted to eliminate external factors that would potentially influence tear production in the present study. The camels were restrained gently and those that required excessive restraint were excluded because the resulting stress may have influenced the sympathetic and/or parasympathetic components that innervate the lacrimal gland.³⁷ All measurements in the current study were obtained between 8:00 a.m. and 11:00 a.m., to avoid variation arising from diurnal rhythm effects on tear production.^{38–40} Furthermore, our study measurements

were conducted indoors in an environment with a similar temperature and humidity at the time of each measurement to avoid any external factors (i.e., sun rays, wind) that could potentially stimulate reflex tears.

The effects of sedation on STT should be considered while performing ophthalmic examinations and procedures in animals. Several studies conducted on a variety of species have demonstrated that tear production is usually reduced by sedation and/or general anesthesia.^{41–43} Therefore, chemical sedation and local nerve blocks were not employed in our study.

In the present investigation, Schirmer tear test results displayed satisfactory repeatability, especially in mature camels, where the interassay CV% values were small (STT I, 6.7 ± 3.1 and 4.2 ± 1.6 and STT II, 5.5 ± 3.1 , 4.1 ± 1.7 for right and left eye, respectively). Although there was significant increase in interassay CV% in immature camel calves compared with other age groups, the results of interassay CV were still at an acceptable level (<15%).

A limitation of the present study was its small sample size; therefore, percentiles were reported in an effort to better describe the range of STT values in clinically normal dromedary camels. We obtained one measurement for STT I and one for STT II per camel eye per test day; therefore, intra-assay CVs could not be calculated. Also, one investigator performed all STT measurements; thus, the reproducibility of measurements between investigators (i.e., interinvestigator CV) could not be calculated. An additional limitation is that we acquired STT measurements under standardized environmental conditions; therefore, further studies are necessary to compare STT measurements obtained under different seasonal, environmental, and management conditions.

In conclusion, this pilot study provided new data that serve as a set of initial values for tear production in normal dromedary camels. Both STT I and STT II were well tolerated in all camels, without a need for chemical restraint or nerve block, and could be used routinely for the ophthalmic examination of camels. The interassay CVs for tear production measurements indicated that STT is a practical and accurate method for determining tear production in dromedary camels. Knowledge of normal STT values in *C. dromedarius* will assist veterinary practitioners with evaluation of corneal pathology and diagnosis of tear deficiency syndromes.

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