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Assessment of Tear Film in Arabian Horses with a Portable Device Compared to Human's Healthy Eyes

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Abstract

THE stability of the tear film is essential to maintain healthy vision in animals and humans. Therefore, tear film should be assessed occasionally to detect any abnormalities, particularly those living in an environment with high temperature and humidity. The current study assessed the non-invasive tear breakup time (NITBUT), tear meniscus height (TMH), and lipid layer pattern (LLP) in Arabian horses and compared them to those of humans. The study involved a total of 94 Arabian horses and 94 humans with healthy eyes. Each subject's right eye tear film parameters were evaluated using the EASYTEAR View+, with a 5-minute interval between each test. The same examiner performed each test three times. The statistical analysis revealed significant differences in the NITBUT (P = 0.001) and LLP (P = 0.010) scores between horses and humans. The LLP analysis indicated that grade E or 5 (variable colors, lipid layer thickness, LLT, = 90–140 nm) was predominant in horses (N = 52, 55.3%). On the other hand, grade D or 4 (dense white-blue layer; around 80 nm) was common in humans with healthy eyes (N = 48, 51.1%). No significant difference (P = 0.191) in the TMH scores between horses and humans was found. In conclusion, horses have a longer tear breakup time and thicker lipid layer height than humans with healthy eyes. Their tear meniscus height is similar to that of healthy humans.

Keywords: Arabian horses; Ocular tear film; Non-invasive tear breakup time; Lipid layer patterns; Tear meniscus height.

Introduction

Tear film quality and stability in animals and humans are essential to maintain healthy vision [1]. The structure of the tear film is highly complex and mainly contains water, proteins, lipids, mucins, and electrolytes [2]. The dysfunction of the tear film leads to many ocular disorders, such as dry eyes [3,4]. The two most common types of dry eye result from aqueous deficiency and a high rate of tear evaporation [5]. Aqueous deficient dry eye is more common in horses due to nerve dysfunction or trauma [6]. In comparison, the evaporative dry eye in horses results from meibomian gland dysfunction (MGD) and irritates the tear film [7].

The MGD harms the lipid layer's quantity and quality in the tear film [8]. For example, it leads to several ocular surface discomforts, such as evaporative dry eye [9,10]. The prevalence of MGD globally in humans was 35.8% [11] and even higher (70%) in dogs having ocular disorders [12]. In horses, MGD can cause persistent epithelial defects and dry eye [13]. Although non-healing corneal ulceration is more common in dogs than in horses, it should be considered when evaluating an older horse [14]. Therefore, the thickness of the lipid layer plays an essential role in keeping tear film healthy. The lipid layer reduces the tear evaporation rate and moistens the tear film. It distributes tear film evenly over the cornea. The thinner the lipid layer, the more the tear film destabilizes due to the increased osmolarity and evaporation.

The tear film assessment is challenging; therefore, different tests and devices should be used [15–17]. Each test assesses a parameter that detects tear volume, stability, osmolarity, evaporation, and quality [18–23]. The tear ferning (TF) test is an excellent tool for assessing tear film in humans and

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animals. The TF test was used to evaluate the quality of tears in horses [24], dogs [25], camels [26], cats [27], and rats [28]. The results obtained from the IF test support the findings from other tests. The TF has good repeatability; however, fern (i.e., crystals) formation is highly dependent on conditions (e.g., temperature, humidity, and dust) [29].

Since the association between tear film parameters is poor, a combination of tests is required to detect abnormalities in the tear film. Some tests need a long time or anesthesia to proceed (e.g., the Schirmer test), and others could be less comfortable. Therefore, using a single device for the noninvasive assessment of different tear film parameters is highly recommended. Indeed, EASYTEAR View+ was used to assess tear film parameters in humans [30]. The current research reports for the first time the noninvasive assessment of noninvasive tear breakup time (NITBUT), tear meniscus height (TMH), and lipid layers patterns (LLP) in horses and compares them to those of humans. It was hypothesized that the lipid layer thickness and NITBUT are greater in horses than in humans due to their living in high-temperature conditions.

Material and Methods

Horses and human subjects with healthy eyes

Ninety-four Arabian horses (48 males and 46 females; mean ± SD = 5.7 ± 3.1 years) were recruited randomly from different stables. The horses' sample size was calculated with a confidence level of 85% and a significance level of 0.05. The horses underwent a thorough ocular examination by a veterinary ophthalmologist using a full slit lamp. Additionally, a veterinarian conducted a systemic health check on all horses. Only healthy horses with no ocular disorders or diseases were included in the study, while the few that did not meet these criteria were excluded. Every precaution was taken to guarantee that the horses were not subjected to any degree of harm or trauma during the experiments. No topical anesthetics were placed on the ocular surface either prior to or throughout the evaluations of the tear film. Moreover, the study included 94 humans with healthy eyes (45 males and 49 females; mean ± SD = 25.9 ± 5.2 years) for comparison. A slit lamp was used to check for abnormalities within the eyelids, conjunctiva, pupil, cornea, iris, and retina in human subjects. The human participants completed the ocular surface disease index (OSDI). Individuals with ocular abnormalities and OSDI scores above 13 were excluded [31]. It was important to compare tear film parameters in horses with those in humans to understand how they adapt to high temperatures.

The tear film parameters of each subject's right eye were evaluated using EASYTEAR View+ (Figure 1). There was a 5-minute gap between different tests. The experiment involved testing horses and human subjects under specific conditions. The horses were tested at a barn, and the temperature ranged between 20 and 25 °C between 9 and 12 am. The human subjects were tested at a clinic with a temperature range of 20 to 22 °C between 9 and 12 am. The humidity was between 15 and 20% in the barn and the clinic. The same examiner conducted the tests three times for both horses and humans, and the mean scores were calculated. The principles of the Declaration of Helsinki were followed when providing treatment to the individuals who took part in the study. The methods used within the study were implemented according to the relevant guidelines and regulations.

EASYTEAR View+

EASYTEAR View+ is a portable device used to diagnose dry eye. It evaluates tear film parameters in animals and humans using white, blue, and infrared LED lighting. The device can assess the regularity of the cornea and evaluate fluorescein staining of the anterior segment. It also measures NITBUT and TMH, visualizing the interference of the tear film with the lipid phase. A single examiner performed the NITBUT, TMH, and LLP measurements in triplicate and then calculated average scores. Five minutes between consecutive tests were allowed as a gap [30].

Non-invasive tear breakup time (NITBUT) test

The duration of the time, measured in seconds, between a blink and a dry spot appearance in the tear film is referred to as NITBUT. A NITBUT result of less than 10 seconds indicates dry eye [32].

Tear meniscus height (TMH) test

The TMH height (in millimeters) is the triangular cross-section between the margin of the lower lid and the cornea. A height lower than 0.2 mm is considered dry eyes [33].

Lipid layer patterns (LLP) test

The classification of the lipid layer into five different categories is a quick and precise process. Grade A (LLT = 13–15 nm; gray appearance), grade B (LLT = 30–50 nm; more compact), grade C (LLT = 50–80 nm; gray waves), grade D (LLT = around 80 nm; dense white-blue layer), and grade e (LLT = 90–140 nm; variable colors) were assigned to the LLP for horses and humans [34]. The grades A–E were replaced by a score of 1–5, respectively, to allow statistical analysis as previously reported.[30]

Statistical analysis

The SPSS software (version 22) developed by IBM Software was utilized to analyze the data. The Kolmogorov–Smirnov test ($P < 0.05$) determined the non-normal distribution of the data. Therefore, the Mann–Whitney U test was employed to analyze the data in both groups. Spearman's correlation coefficient (r) tested the association between different parameters [35]. Meanwhile, the Wilcoxon signed-rank test was employed to investigate the significance of any differences between other parameters within the same group. The mean scores were calculated and represented as the median and interquartile range (IQR).

Results

The median scores for the NITBUT, TMH, and LLP in horses and healthy-eye humans are given in Table 1. The data showed significant differences (Mann–Whitney U test) in the median scores of the NITBUT (P = 0.001) and LLP (P = 0.010) scores between horses and subjects with healthy eyes. On the other hand, there was no significant difference in TMH between the horses and human groups (Mann-Whitney U Test, P = 0.191). The NITBUT score was significantly longer for horses compared to normal humans. Figure 2 represents the side-by-side boxplots for the NITBUT and LLP in horses and healthy-eye humans.

The LLP analysis indicated that grade 5 or E (variable colors, LLT = 90–140 nm) was predominant in horses (N = 52, 55.3%). On the other hand, grade 4 or D (dense white-blue layer; ~80 nm) was typical in humans with healthy eyes (N = 48, 51.1%). Figure 3 shows the LLP grades for the horses and human groups. Figures 4 and 5 display representative images of the common lipid layer patterns in horses and humans, respectively.

The statistical analysis showed a medium correlation (r = 0.361, P < 0.001) between the age and TMH scores in horses. In addition, it indicates a weak correlation (r = 0.273, P < 0.010) between the TMH and NIBUT scores. As age increased, TMH score increased.

Discussion

Assessment of various tear parameters using a single device is very convenient and noninvasive. The current research is the first report to assess the tear film parameters in horses using a single device. The measurement of the NITBUT, TMH, and LLP indicated that horses have scores similar to those of humans with healthy eyes. EASYTEAR View+ showed that horses have significantly longer NITBUT and thicker lipid layers than humans with healthy eyes. These findings have significant implications for veterinary and optometric practice. They provide a basis for understanding and diagnosing ocular disorders in Arabian horses and can inform the development of new treatment strategies.

Various factors lead to differences in the scores of tear film parameters between horses and humans. The tear film thickness is approximately 93 μm in horses, which is thicker than that for humans (7 μm) [36]. In addition, the tear flow rate and tear volume are much higher in horses (34 μL/min and 234 μL, respectively) compared to humans (1.2 μL/min and 7 μL, respectively) [36]. The status of the tear film is significantly affected by humidity and temperature. Horse tears are less acidic (pH = 8.3) compared to those of humans (pH = 6.5–7.6) [37,38]. The concentration of calcium cations plays a role in stabilizing the tear film in horses. A high calcium level causes its precipitation and the failure of the tear film function [39]. Maintaining the health of the vision system is dependent on the concentration of proteins present in tears. Tears collected from horses showed a very high level of proteins (13.7 ± 4.0 mg/mL) compared to rabbits (10.2 ± 3.5 mg/mL), cows (5.8 ± 2.2 mg/mL), and dogs (2.6 ± 1.0 mg/mL) [40]. On the other hand, the concentration of protein in human tears varied from 6 to 10 mg/mL [41].

Recent reports indicated that the LLP among animals varied from that of humans [42,43]. Significant differences (P < 0.01) were found in the LLP among horses, cats, dogs, and rabbits. Grade E was found to be predominant in horses (55.3%) compared with grade D in cats (44.4%), grade B in dogs (37.3%), and grade A in rabbits (46.7%) [42,43]. Clearly, horses are thicker than other animals. Horses have bigger eyes compared to other animals, such as cats, dogs, and rabbits, and the tear evaporation rate would be expected to be high unless the lipid layer thickness is large. However, there was no significant difference in the TMH scores among Arabian horses compared with those for cats [42].

Healthy horses have a tear film osmolarity (283.5 Osm/L) that is similar to humans (282.5 Osm/L) using a vapor pressure osmometer [44]. However, horses have a much lower tear osmolarity compared with dogs (355.5 Osm/L), cats (328.5 Osm/L), and rabbits (375.8 Osm/L) [45–46]. The osmolarity of the tear film is highly dependent on various factors such as tear composition, evaporation, and production. In addition, the device used to measure osmolarity has a role to play. Hyperosmolarity can result from high tear evaporation (i.e., evaporative dry eyes) [47]. Horses have similar sodium (monovalent) levels in the tear film and serum.[47] In comparison, potassium in the tear film of horses has a higher concentration than that in the serum by 4.75 times. The concentration of divalent cations (e.g., calcium and magnesium) was much higher in the tear film of horses compared to rabbits and humans [48].

The TF test was used to evaluate the quality of tears collected from the right and left eyes of 30 horses [24]. The TF grades were Type I and II (normal eyes), based on the Rolando grading scale, for most eyes (N = 49; 81.7%). The TF grade for tears collected from 18.3% of the eyes showed Type III (dry eyes) [24]. The TF grades of the right eye are not associated with that of the left one. In contrast to humans, the TF grades do not correlate with the age of horses. According to a previous study, adults over

50 commonly have a Type III TF grade at 63%, while children have it at 17% [49].

A future study is still needed to assess the tear film parameters of other animals that live in different environments and compare them to those of horses. In addition, evaluating the relationship between lipid layer thickness and tear film parameters such as evaporation, ferning, and osmolarity of tears is essential. Moreover, the Schirmer tear test needs to be performed to confirm sufficient tear quantity.

Conclusion

A portable device was successfully used to assess different tear film parameters in Arabian horses and humans. Arabian horses have significantly longer tear breakup time and thicker lipid layer height than humans with healthy eyes. No significant difference was observed in the height of the tear meniscus between Arabian horses and humans.

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Funding statement

King Saud University.

Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

The study was approved by the King Saud University IRB Committee (E-22-6562). The relevant guidelines and regulations were followed in the tests.

TABLE 1. Median (IQR) scores for the NITBUT, TMH, and LLP in horses compared to human's healthy eyes.

Parameter	Horses (*N* = 94)	Healthy-eye humans (*N* = 94)	*P*-value
NITBUT (s)	21.3 (14.0)	12.0 (7.0)	0.001*
TMH (mm)	0.16 (0.06)	0.15 (0.10)	0.191
LLP	5 (1)	4 (1)	0.010*

*Significant difference (Mann–Whitney U test).

Fig. 1. The use of EASYTEAR View+ to detect tear film parameters in horses.

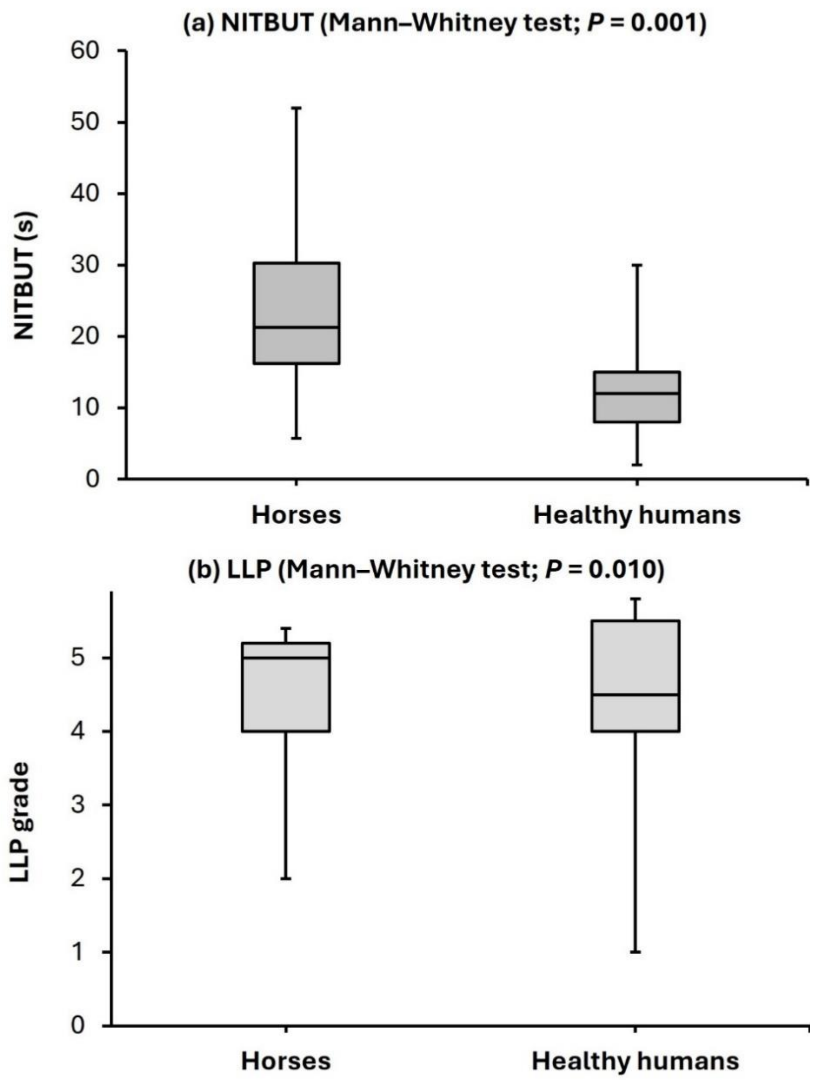

Fig. 2. Side-by-side boxplots of (a) the NITBUT scores (s) and (b) LLP grades in horses and humans..

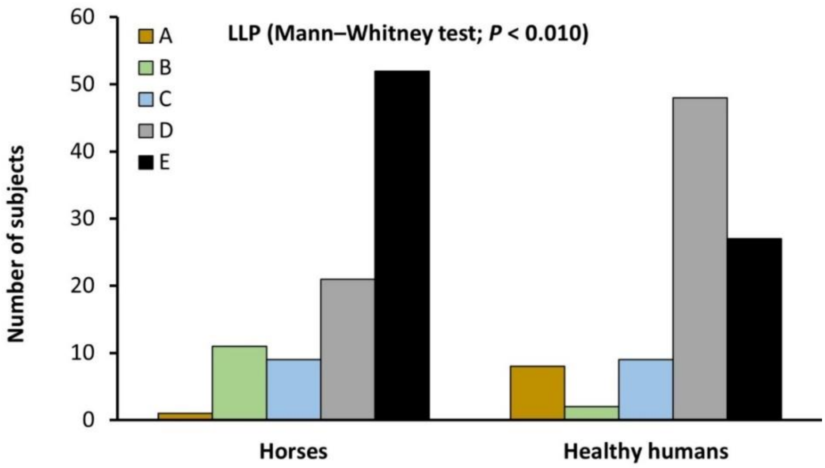

Fig. 3. LLP representation in horses and humans. Grade 1 or A (LLT = 13–15 nm; gray appearance), grade 2 or B (LLT = 30–50 nm; more compact), grade 3 or C (LLT = 50–80 nm; gray waves), grade 4 or D (LLT = around 80 nm; dense white-blue layer), and grade 5 or e (LLT = 90–140 nm; variable colors).

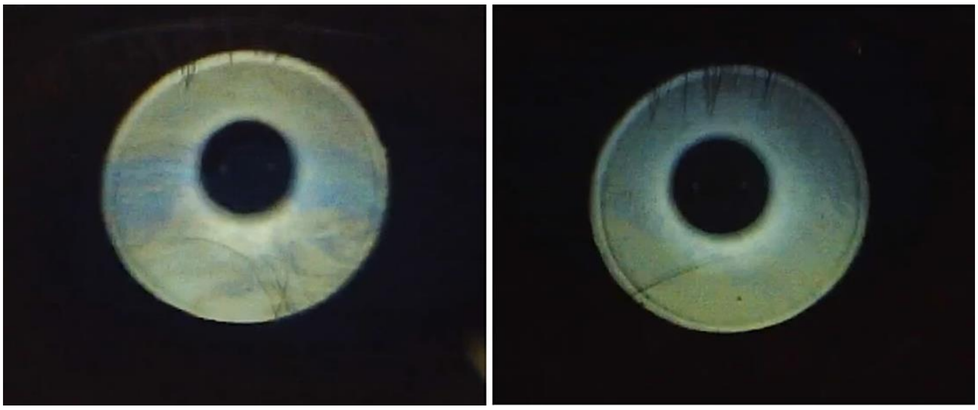

Fig. 4. Representative images of the common lipid layer patterns in horses.

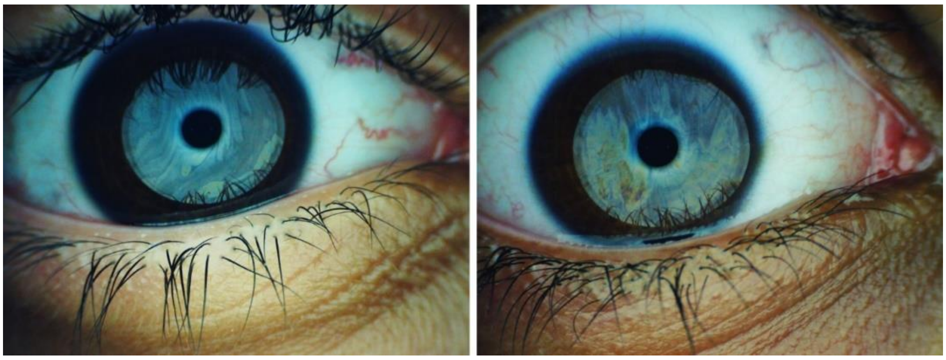

Fig. 5. Representative images of the common lipid layer patterns in humans.

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تقييم الشريط الدمعي في الخيول العربية باستخدام جهاز محمول مقارنةً بعيون الإنسان السليمة

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الملخص

إن ثبات الشريط الدمعي ضروري للحفاظ على صحة الرؤية لدى الحيوانات والبشر. لذلك، يجب تقييم الشريط الدمعي من حين لآخر للكشف عن أي ظواهر غير طبيعية، وخاصة تلك التي تعيش في بيئة ذات درجة حرارة ورطوبة عالية. قامت الدراسة الحالية بتقييم وقت تفكك الدموع وارتفاع هلال الدموع ونمط الطبقة الدهنية في الخيول العربية ومقارنتها بتلك الموجودة لدى البشر. كانت الفرضية هي أن بتقييم وقت تفكك الدموع وسمك الطبقة الدهنية أكبر في الخيول منها لدى البشر بسبب معيشتهم في ظروف درجات حرارة عالية. شملت الدراسة ما مجموعه 94 حصانًا عربيًا و94 إنسانًا يتمتعون بعيون سليمة. تم تقييم معلمات طبقة الدموع في العين اليمنى لكل مشارك باستخدام جهاز محمول بفاصل زمني مدته 5 دقائق بين كل اختبار. قام نفس الفاحص بإجراء كل اختبار ثلاث مرات. كشف التحليل الإحصائي عن اختلافات جوهرية في وقت تفكك الدموع وسمك الطبقة الدهنية بين الخيول والبشر. لم يتم العثور على فرق كبير في ارتفاع هلال الدموع بين الخيول والبشر.

الكلمات الدالة: الخيول العربية، شريط الدمع العيني، وقت تفكك الدموع، أنماط الطبقة الدهنية، ارتفاع هلال الدموع.