Ocular findings in Quarter Horses with hereditary equine regional dermal asthenia

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EQUINE

Objective—To compare ocular structures of Quarter Horses homozygous for hereditary equine regional dermal asthenia (HERDA) with those of Quarter Horses not affected by HERDA (control horses) and to determine the frequency of new corneal ulcers for horses with and without HERDA during a 4-year period.

Design—Cohort study of ocular structures and retrospective case series of horses with and without HERDA.

Animals—The cohort portion of the study involved 10 Quarter Horses with HERDA and 10 Quarter Horses without HERDA; the retrospective case series involved 28 horses with HERDA and 291 horses without HERDA.

Procedures—Ophthalmic examinations, Schirmer tear tests, tonometry, corneal pachymetry, histologic examinations, and scanning electron microscopy (SEM) were performed in cohorts of Quarter Horses with and without HERDA. Records were reviewed to determine the incidence of corneal ulcers in horses with and without HERDA during a 4-year period.

Results—Corneal thickness of horses with HERDA was significantly less than that of control horses, but tear production of horses with HERDA was significantly greater than that of control horses. Results of SEM revealed zones of disorganized, haphazardly arranged collagen fibrils in corneas of horses with HERDA that were not evident in corneas of control horses. The incidence of corneal ulcers was significantly greater for horses with HERDA than for horses without HERDA during the 4-year period.

Conclusions and Clinical Relevance—Alterations in corneal thickness, arrangement of collagen fibers, and incidence of corneal ulcers indicated that abnormalities in horses with HERDA were not limited to the skin. (*J Am Vet Med Assoc* 2010;237:304–310)

The condition HERDA, also known as hyperelastosis cutis or EDS, is an autosomal recessive disorder of horses that results in fragile skin that tears easily and has impaired healing.[1–6] There are similar conditions in humans,[7] cattle,[8] cats,[9] dogs,[9,10] rabbits,[11] and other species.[11] The HERDA condition is a generalized connective tissue disorder characterized by alterations in collagen microarchitecture.[6] Collagen defects allow for separation between the deep and superficial dermis, which results in a layer of epidermis and superficial dermis that is not tightly adhered to the deeper layer of the dermis and creates a loose or stretched appearance.[1,3,6] Lesions often are the result of minimal trauma, and wounds heal poorly.[1–6]

The HERDA condition is becoming increasingly prevalent and important in Quarter Horses. In a recent study,[12] the overall carrier frequency for HERDA within the American Quarter Horse population was 2%. The cutting horse industry is the most seriously affected of the Quarter Horse disciplines, with an estimated carrier frequency of 28%.[12,13]

Abbreviations

EDS	Ehlers-Danlos Syndrome
HERDA	Hereditary equine regional dermal asthenia
IOP	Intraocular pressure
SEM	Scanning electron microscopy

Humans are affected by a group of similar hereditary disorders collectively known as EDS. The EDS condition may result from any number of mutations that affect genes responsible for collagen formation and processing; thus, clinical effects of the disorder vary according to the type of collagen or specific processing step affected by the mutation. A detailed classification scheme has been developed for the various forms of EDS in humans.[14] In horses, HERDA has been traced to a mutation in the gene encoding for cyclophilin B, which is a cis-trans isomerase thought to facilitate procollagen folding.[15] Similarities between HERDA and EDS are not limited to clinical signs and common lineage. Other consistent findings include an increase in the urinary

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deoxypyridinoline-to-pyridinoline collagen cross-link ratio.[16] In humans, EDS type VIA is characterized by scleral fragility and rupture of the globe, which is often in conjunction with other ocular abnormalities that include a blue sclera, keratoconus, corneal thinning, and corneal perforations.[17–24] Similar ocular abnormalities have also been identified in cats[9] and dogs[9,10] with EDS-like syndromes. Both dogs and cats can develop the ocular changes of microcornea, sclerocornea, lens luxation, and cataracts.[9,10]

An ocular component of HERDA has yet to be reported in horses. The purpose of the study reported here was to compare corneal thickness, corneal microarchitecture, and results of ophthalmic examination for Quarter Horses with and without HERDA and to determine the frequency of new corneal ulcers for horses with and without HERDA that were a part of the equestrian programs at our university during a 4-year period. We hypothesized that collagen abnormalities that affect the skin of horses with HERDA would also be manifested in the ocular tissues, which would result in corneal thinning and an increased incidence of corneal ulcers.

Materials and Methods

The study comprised 2 portions. A prospective cohort evaluation and a retrospective case series were performed.

Prospective cohort evaluation—This portion of the study involved the use of 20 Quarter Horses. Horses were cared for and the study was conducted in a manner consistent with guidelines established by the National Institutes of Health[25] and the Animal Welfare Acts. Experimental protocols were approved by the Mississippi State University Institutional Animal Care and Use Committee.

All selected horses were a part of the equestrian programs at Mississippi State University. Each mature (≥ 2 years old) Quarter Horse homozygous for HERDA that could be compared with a Quarter Horse without HERDA that had the same sex and similar age was included in the study. Ten Quarter Horse mares with HERDA that were between 2 and 10 years of age met the inclusion criteria. Thirty-four Quarter Horse mares without HERDA that were between 2 and 10 years of age met the inclusion criteria. A list of the horse identification numbers of the 34 mares was compiled, and a control group of 10 mares without HERDA was then chosen by random selection from the list. The HERDA genotypic status of each horse was confirmed via DNA testing for the cyclophilin B mutation, which is the genetic basis for the disorder in horses.[15]

Ocular examination and Schirmer tear test

All horses were evaluated for epiphora, blepharospasm, facial symmetry, and photophobia. Corneal clarity and the anterior chamber, lens, posterior chamber, retina, and optic disc were evaluated by a board-certified veterinary ophthalmologist (WWM). All examinations were performed during a 2-day period. Horses were chemically restrained by administration of xylazine hydrochloride[a] (0.1 mg/kg [0.045 mg/lb], IV) and butorphanol tartrate[b] (0.01 mg/kg [0.0045 mg/lb], IV). Auriculopalpebral and supraorbital nerves were anesthetized with 2.0 mL of 2% mepivacaine.[c] Eyes of all horses were evaluated with diffuse and focal illumination by use of a portable slit lamp[d] and an indirect ophthalmoscope.[e] Following ophthalmic examination but before application of dilatory medications to the eyes, a Schirmer tear test[f] was performed on both eyes. The pupils then were dilated by topical application of 0.3 mL of tropicamide[g] to enable examination of the fundus. Prior to corneal ultrasonography, all corneas were anesthetized by topical application of 0.3 mL of 0.5% proparacaine.[h]

Corneal pachymetry

Corneal thickness was measured by use of a 20-MHz A-scan ultrasound probe[i] applied to the surface of the central region of the cornea. Velocity of sound transmitted through the cornea was set at 1,640 m/s. The transducer was placed perpendicular to the central corneal region. Three measurements of the central corneal region were collected from each eye of each horse.

Measurement of IOP

The IOP was measured in each eye of each horse with a tonometer[j] used in accordance with the manufacturer's recommendations. Three measurements were obtained for each eye.

Histologic examination and SEM

Two corneal samples for histologic examination and SEM were collected from 6 of the 10 Quarter Horses with HERDA immediately after the horses were euthanatized by administration of an overdose of pentobarbital. These horses were euthanatized because of severe skin lesions or other medical problems. Corneas were collected from eyes of 4 euthanized HERDA horses with no previous history of corneal disease or evidence of corneal disease during ophthalmic examination. One cornea was from an eye with a corneal ulcer that had healed 23 months prior to euthanasia of the horse. One cornea was from an eye with anterior uveitis that had resolved 12 weeks prior to euthanasia of the horse. Corneal samples were collected from 2 Quarter Horses without HERDA immediately after they were euthanatized by administration of an overdose of pentobarbital; both horses were euthanatized for reasons unrelated to this study. Both of these horses had no history of corneal disease or evidence of corneal disease during ophthalmic examination.

A portion of each corneal sample was placed in neutral-buffered 10% formalin[k] and was processed routinely and stained with H&E stain[l,m] for histologic examination. A portion of each corneal sample was also immersed in McDowell fixative (4% formaldehyde plus 1% glutaraldehyde). Following fixation, the samples were rinsed, postfixed in buffered 2% osmium tetraoxide,[n] and dehydrated in a graded series of ethanol. Corneal samples were dried further by use of hexamethyldisilazane[o] before finally being allowed to air-dry. Dried corneal samples were mounted on aluminum stubs with carbon paste, coated with gold-palladium by use of a sputter coater,[p] and viewed on a scanning electron microscope[q] at 5 kV.

Retrospective case series—This portion of the study involved examination of records of the horses that were a part of the equestrian program at Mississippi State University during the period from January 2003 through December 2006. Records were reviewed to determine the numbers of horses with and without HERDA (on the basis of phenotypic appearance) and the number of horses in each group that developed new corneal ulcers. Horses with and without HERDA were primarily maintained in small groups in pastures throughout the year. Management practices and veterinary care were the same for both groups, and horses from both groups were often housed together in the same pastures. The number of horses in each group fluctuated over time as a result of deaths, birth of new foals, sale of weanlings and adult animals, and donations of additional horses to the program; thus, the number of horses that were a part of the program and at risk for developing a corneal ulcer was calculated on a monthly basis for the horses of both groups. The total number of horse-months for a group was the sum of the numbers of months that each horse was present and at risk for developing a corneal ulcer during the study.

Statistical analysis—For the prospective cohort evaluation, corneal thickness, IOP, and Schirmer tear test measurements were evaluated by use of the Kolmogorov-Smirnov test to determine whether the data were normally distributed for each group. Corneal thickness and IOP were recorded as the mean of the values obtained during examination of each eye. Measurements for each variable were evaluated for both groups to determine whether there was a significant difference between the left and right eyes. For data that were normally distributed, a paired *t* test was used, whereas for data that were not normally distributed, the Wilcoxon signed rank test was used. When no significant difference was found between the left and right eyes for a variable, the value for each horse was calculated as the mean of the values for the left and right eyes. For data that were normally distributed, a 2-sample *t* test was used to detect significant differences between groups. When data were not normally distributed, the Wilcoxon rank sum test was used to detect significant differences between groups. The effect of age on corneal thickness was evaluated for each group by use of linear regression.

For the retrospective case series, the total number of horse-months was calculated for the horses at risk of developing a corneal ulcer in both groups (ie, with and without HERDA) during the period from January 2003 through December 2006. The incidence of corneal ulcers during the period was expressed as the number of new corneal ulcers/1,000 horse-months for each group during the study.[26] The Fisher exact test was used to detect differences in incidence rate between groups. A commercially available statistical program[r] was used for all statistical tests. Differences were considered to be significant at values of $P < 0.05$.

Results

Prospective cohort evaluation—The HERDA group consisted of 10 Quarter Horse mares between 2 and 10 years of age (mean ± SD, 4.6 ± 2.3 years). The control group consisted of 10 Quarter Horse mares between 3 and 10 years of age (mean ± SD, 8.1 ± 2.1 years).

Ophthalmic examination

Ophthalmic examination of the 10 control Quarter Horses revealed no abnormalities. Results of ophthalmic examination of the 10 Quarter horses with HERDA revealed lesions in 3 horses. One mare had pigment dispersion on the anterior lens capsule of the right eye, a second mare had a chorioretinal scar of the right eye, and a third mare had a multifocal anterior capsular cataract of the left eye. No clinically detectable abnormalities were evident in the cornea of any of the horses with HERDA.

Corneal pachymetry

Data for corneal thickness measurements did not have a normal distribution (**Figure 1**). Therefore, statistical analysis to detect differences between left and right eyes was conducted by use of the Wilcoxon signed rank test. There was no significant difference in corneal thickness between the left and right eyes of either group, so the mean of the values of the left and right eyes was used for further statistical analysis. No significant association was detected between age and corneal thickness for either group of horses. The Wilcoxon rank sum test was used to determine differences in corneal thickness between the Quarter Horses with and without HERDA. Our hypothesis was that the corneas of the horses with HERDA would be thinner than those of the horses without HERDA; therefore, statistical analysis was conducted by use of a 1-tailed test. Corneal thickness of Quarter Horses with HERDA (median, 735 µm; interquartile range, 100 µm) was significantly ($P = 0.043$) less than that of Quarter Horses without HERDA (median, 800 µm; interquartile range, 45 µm).

Although no corneal abnormalities were detected in any of the 20 Quarter Horses at the time of ophthalmic examination, evaluation of the medical records revealed that 1 horse with HERDA had a corneal ulcer that healed 12 months prior to the ophthalmic examination and that a second horse with HERDA had an episode of bilateral anterior uveitis with corneal edema

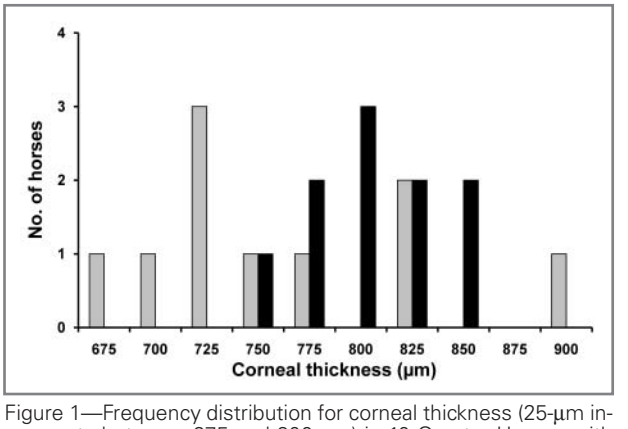

Figure 1—Frequency distribution for corneal thickness (25-µm increments between 675 and 900 µm) in 10 Quarter Horses with HERDA (gray bars) and 10 Quarter Horses without HERDA (black bars).

that resolved with medical management 4 weeks prior to the ophthalmic examination. Interestingly, the corneal thickness of these 2 horses (810 µm and 900 µm, respectively) was greater than that of the other horses with HERDA in the study. Had the data for these 2 horses been excluded from analysis (ie, analysis of data for horses without a history of ocular disease), the differences in corneal thickness between the 8 remaining horses with HERDA (median, 720 µm; interquartile range, 53 µm) and the 10 horses without HERDA (median, 800 µm; interquartile range, 45 µm) would have been even more apparent ($P = 0.007$).

Schirmer tear test

Tear production of Quarter Horses with HERDA was significantly ($P < 0.001$) greater than that of Quarter Horses without HERDA (mean ± SD, 32 ± 4 mm and 25 ± 4 mm, respectively). There was no significant difference in tear production between the left and right eyes of either group.

IOP

The IOP of Quarter Horses with HERDA was not significantly different from that of Quarter Horses without HERDA (mean ± SD, 16 ± 4 mm Hg and 18 ± 2 mm Hg, respectively). There was also no significant difference in IOP between the left and right eyes of either group.

Histologic examination and SEM

Histologic examination of transverse sections of the formalin-fixed corneas did not reveal obvious abnormalities. Some artifactual separation of the collagen lamellae of the substantia propria was attributed to formalin fixation. Corneal samples for SEM were oriented transversely, which allowed examination from corneal epithelium across the lamellae of the substantia propria to the corneal endothelium. Quarter Horses without HERDA had lamellae of densely packed, uniformly oriented collagen fibers that were occasionally spanned by individual fibrils at points of artifactual separation (**Figure 2**). Quarter Horses with HERDA had variable degrees of multifocal disarray of this anatomically normal lamellar pattern. Although uniformly oriented in a manner similar to that seen in the Quarter Horses without HERDA, the lamellae of horses with HERDA were intermittently replaced by zones of disorganized, haphazardly arranged, tangled collagen fibrils (**Figure 3**). Curls, twists, and complex haphazard interconnections were often in distinct contrast to adjacent uniformly oriented collagen lamellae. The disarray in the lamellar pattern was evident in the corneas of all horses with HERDA. Similar disarray of the lamellar pattern was prominent in the cornea of the Quarter Horse with HERDA that had an episode of uveitis and in the cornea of the Quarter Horse with HERDA that had a healed corneal ulcer (**Figure 4**).

Retrospective case series—Evaluation of records indicated that during the 4-year study period, there were 16 adult horses and 12 foals with HERDA present as a part of the equestrian programs for differing amounts of time. The horses with HERDA were all Quarter Horses. The adults were between 2 and 10 years of age. Sex distribution was 12 mares, 2 geldings, and 2 stallions for the adults, and there were 7 filly and 5 colt foals. The 28 horses with HERDA were at risk of developing a corneal ulcer during the study for a total of 840 horse-months; the horses with HERDA were a part of the equestrian programs and in the study for a mean of 30 months each. During the study, there were 135 adult horses and 156 foals without HERDA present as a part of the equestrian programs for differing amounts of time. Adult horses without HERDA consisted of 114 adult Quarter Horses, 11 Thoroughbreds, and 10 Hackney ponies. Adults were between 2 and 24 years of age. Sex distribution was 130 mares and 5 stallions. Foals without HERDA consisted of 130 Quarter Horses, 22 Thoroughbreds, and 4 Hackney ponies. There were 82 filly and 74 colt foals. The 291 horses without HERDA were at risk of developing a corneal ulcer during the study for a total of 7,416 horse-months; the horses without HERDA were a part of the equestrian programs and in the study for a mean of 25 months each.

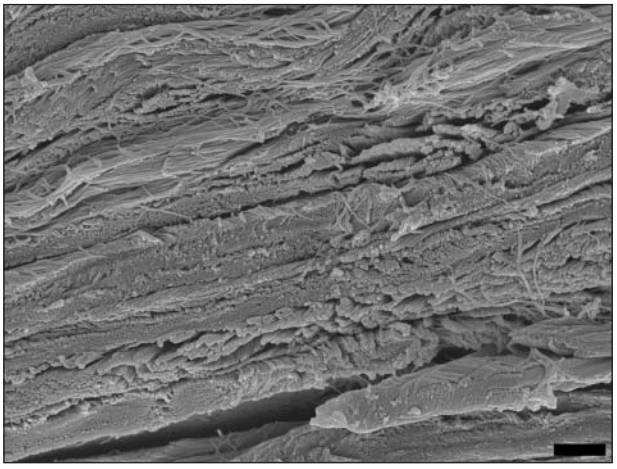

Figure 2—Scanning electron micrograph of a section of cornea obtained from a Quarter Horse without HERDA. Notice the uniform lamellar layers of collagen fibers in a parallel arrangement in the corneal substantia propria. Sputter coated with gold-palladium. Bar = 1 µm.

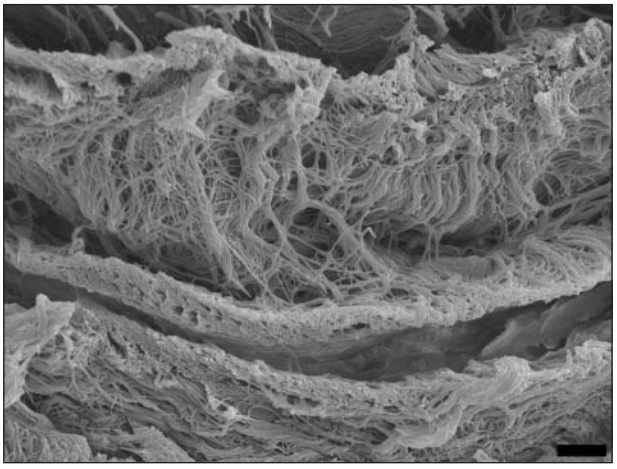

Figure 3—Scanning electron micrograph of a section of cornea obtained from a Quarter Horse with HERDA. Notice the disorganized, haphazardly arranged, tangled collagen fibrils in intermittent lamellae in the corneal substantia propria. This horse had no history of corneal disease. Sputter coated with gold-palladium. Bar = 1 µm.

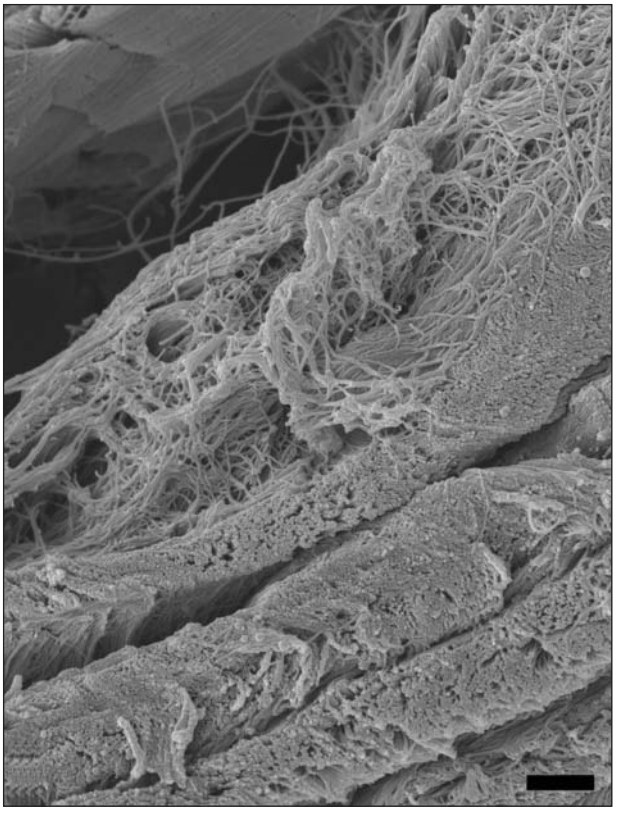

Figure 4—Scanning electron micrograph of a section of cornea obtained from a Quarter Horse with HERDA. The horse had a corneal ulcer that healed 23 months before the horse was euthanatized. Notice the similarities with the cornea of the horse in Figure 3. Intermittent lamellae in the corneal substantia propria are disorganized and haphazardly arranged. Sputter coated with gold-palladium. Bar = 1 µm.

During the 4-year study period, corneal ulcers developed in 4 horses with HERDA and in 1 horse without HERDA. The incidence of corneal ulcers in horses with HERDA was significantly ($P < 0.005$) greater than that in the horses without HERDA (4.8 corneal ulcers/1,000 horse-months and 0.13 corneal ulcers/1,000 horse-months, respectively).

Discussion

The reported[27] thickness of the equine cornea is 800 to 1,000 µm and is consistent with the median corneal thickness of our control horses (800 µm). The corneas of horses with HERDA were significantly thinner (735 µm) than the corneas of the horses without HERDA in this study and thinner than the reference range reported[27–31] for clinically normal horses. In addition, when the corneal thickness from only the 8 horses with HERDA that did not have a history of corneal disease was analyzed (720 µm), the differences were even more substantial.

Corneal thinning is a finding in several forms of EDS in humans.[17,21] The thinner cornea and defective collagen in patients with EDS are believed to result in biomechanical weakness[21] that results in an increased incidence of keratoconus, keratoglobus, cornea plana, corneal rupture, and ocular fragility.[17,20–24] Similarly, the thinner cornea and defective collagen in horses with HERDA may play a role in the increased incidence of corneal ulcers, when compared with the incidence in horses without HERDA in the retrospective aspect of the study reported here. Thinning in the central region of the cornea in humans without EDS is most often associated with glaucoma.[32] Values for IOP that were within the reference range ruled out glaucoma as a cause of corneal thinning within the horses with HERDA.

Results of SEM revealed areas of collagen disarray in all of the corneas obtained from the horses with HERDA but in none of the corneas obtained from horses without HERDA. The degree of alteration in the collagen microarchitecture varied in severity within the HERDA group and was comparable to the collagen disruption seen within the skin of horses with HERDA.[3–6] In both the skin and cornea, collagen disruption was multifocal and complete destruction of typical stromal architecture was not observed. The altered collagen architecture detected in the skin of horses with HERDA is believed to be the cause of the increase in skin fragility.[6] Interestingly, in addition to the variations in degree of disruption of collagen seen in the cornea and skin, there was considerable variation in the physical manifestation of the skin lesions among the horses with HERDA at Mississippi State University.

Retrospective analysis of the records for horses with and without HERDA at Mississippi State University revealed that the incidence of corneal ulcers was significantly greater for horses with HERDA than for horses without HERDA. The environment and management of both groups of horses were similar. No factor (other than genotype) could be identified to explain the difference in incidence between the groups. Although corneal ulcers in horses are a common and sight-threatening condition, the overall incidence of corneal ulcers in horses has not been determined.[33] In humans without EDS, the reported incidence varies from 27.6 corneal ulcers/100,000 person-years in the United States to as high as 779 corneal ulcers/100,000 person-years in Nepal.[34–37] In Bhutan, 45% of agricultural workers with traumatic corneal abrasions went on to develop corneal ulcers, whereas the other 55% had corneal abrasions that healed without treatment.[36,37] The incidence of corneal abrasions and ulcerations in horses may be higher because of the prominence of the eyes, which may predispose them to traumatic corneal injury.[33] In the horses without HERDA in our study, the incidence of corneal ulcers was 162 corneal ulcers/100,000 horse-years, compared with the incidence of 5,714 corneal ulcers/100,000 horse-years in the horses with HERDA. Precorneal tear film and healthy corneal epithelium provide substantial resistance to adhesion and colonization of pathogenic organisms on the surface of the equine cornea and thereby protect against the development of corneal ulcers.[33] It is likely that clinically normal horses incur mild traumatic corneal abrasions that do not progress to corneal ulcers, which is similar to the situation for the agricultural workers in Bhutan.[36,37] This same degree of mild trauma to the cornea of horses with HERDA may be less self-limiting, similar to the situation for traumatic skin lesions in horses with HERDA,[5,6] and may be more likely to allow pathogens to attach, which ultimately results in corneal ulcers. Al-

terations in precorneal tear film may also play a role in the development of corneal ulcers in horses with HERDA; however, specific analysis of the tear film was not performed in the present study.

Of the other variables measured in the horses with HERDA in our study, a significant increase in tear production[38] was detected. Keratitis, uveitis, corneal ulcers, and conjunctivitis can all result in an increase in tear production.[31,33,39] Less common causes of increases in tear production in horses include structural deformities, allergic diseases, neurologic conditions, and chemical or mechanical irritants.[31,33,39] To our knowledge, an increase in tear production had not been previously associated with HERDA in the absence of observed ocular disease. Analysis of the tears of horses with HERDA was not attempted but may provide additional information that could help explain the increased incidence of corneal ulcers within the horses of the present study. Disarray of collagen fibrils was observed in areas of corneas obtained from horses with HERDA, even in those with no history or current evidence or corneal disease. Such alterations in corneal structure may have been associated with the increase in tear production.

A distinct ocular form of EDS was first recognized in 1970.[14] It is characterized by severe dermal, skeletal, and ocular abnormalities.[14,17–24] One diagnostic test for identifying this type of EDS is an increase in the urinary deoxypyridinoline-to-pyridinoline collagen cross-link ratio.[14] In addition to ocular abnormalities, horses with HERDA have significantly higher urinary deoxypyridinoline-to-pyridinoline ratios than do clinically normal horses.[16]

To our knowledge, an ocular form of HERDA has not been reported previously. However, analysis of the results of our study indicates that horses with HERDA have an increased incidence of corneal ulcers, reduced corneal thickness, and disruption of the typical corneal lamellar pattern, which indicates that abnormal collagen fibers are not restricted to only the cutaneous tissues. The alterations in the arrangement of collagen fibers may be associated with impaired collagen formation or remodeling of the corneal stroma. The 2 mares with corneal disease prior to corneal measurement had the thickest corneas, which possibly may have been related to altered processes involved with collagen production or corneal repair.

Impaired wound healing and tissue repair are important aspects of the HERDA disease process in the skin and are consistent with the cyclophilin B mutation that has been reported as a cause of HERDA.[15] A missense mutation has been identified in equine cyclophilin B; the mutation functions as a cis-trans peptidyl-prolyl isomerase that causes isomerization of proline and thereby facilitates procollagen folding.[15,40] Collagen fibers (particularly collagen type 1 fibers) contain large amounts of proline. Disruption in the collagen arrangement is likely associated with failure of protein folding and is believed to be responsible for the weakness identified within the skin[6] and, potentially, the cornea.

In the study reported here, a decrease in corneal thickness and an increase in tear production were detected in horses with HERDA, compared with results in horses without HERDA. The reduced corneal thickness was not associated with an increase in IOP. Results of SEM confirmed altered arrangement of collagen fibers in the corneas of horses with HERDA. Additionally, horses with HERDA had an increased incidence of corneal ulcers when compared with the incidence for a similar but larger number of horses without HERDA. This information can be particularly useful when managing affected horses with ocular disease. To our knowledge, this is the first report of ocular abnormalities in horses with HERDA and the first report of abnormalities in affected horses that are not related to the skin.

a. AnaSed xylazine sterile solution, 100 mg/mL, Lloyd Inc, Shenandoah, Iowa.
b. Torbugesic butorphanol tartrate, 10 mg/mL, Fort Dodge Animal Health, Fort Dodge, Iowa.
c. Carbocaine-V 2% mepivacaine hydrochloride USP, Pfizer Inc, New York, NY.
d. Kowa SL-15 portable slit lamp, Kowa Co Ltd, Tokyo, Japan.
e. 3.5-volt ophthalmoscope, Welch Allyn, Skaneateles Falls, NY.
f. Sno Strips sterile tear flow, Chauvin Pharmaceuticals Ltd, Romford, Essex, England.
g. Tropicacyl 1% tropicamide ophthalmic solution USP, Akorn Inc, Buffalo Grove, Ill.
h. Proparacaine hydrochloride 0.5% ophthalmic solution USP, Akorn Inc, Buffalo Grove, Ill.
i. Scarab-B 20-MHz ultrasound, Dioptrix, L'Union, France.
j. Tono-pen XL tonometer, Mentor O&O Inc, Norwell, Mass.
k. Neutral-buffered formalin concentrate, Surgipath Medical Industries Inc, Richmond, Ill.
l. SelectTech hematoxylin, Surgipath Medical Industries Inc, Richmond, Ill.
m. SelectTech alcoholic eosin, Surgipath Medical Industries Inc, Richmond, Ill.
n. Osmium tetraoxide, Stevens Metallurgical Corp, New York, NY.
o. Hexamethyldisilazane, Electron Microscopy Sciences, Hatfield, Pa.
p. Polaron E1500 sputter coater, Polaron Equipment Ltd, Watford, Hertfordshire, England.
q. JEOL JSM-6500F scanning electron microscope, JEOL USA Inc, Peabody, Mass.
r. SAS System for Windows, version 9.23, SAS Institute Inc, Cary, NC.

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