Density of corneal endothelial cells, corneal thickness, and corneal diameters in normal eyes of llamas and alpacas

Stacy E. Andrew, DVM; A. Michelle Willis, DVM; David E. Anderson, DVM, MS

Objective—To determine density of corneal endothelial cells, corneal thickness, and corneal diameters in normal eyes of llamas and alpacas.

Animals—36 llamas and 20 alpacas.

Procedure—Both eyes were examined in each camelid. Noncontact specular microscopy was used to determine density of corneal endothelial cells. Corneal thickness was measured, using ultrasonographic pachymetry. Vertical and horizontal corneal diameters were measured, using Jameson calipers.

Results—Values did not differ significantly between the right and left eyes from the same camelid. There was no significant effect of sex on density of corneal endothelial cells or corneal thickness in either species. Mean density of endothelial cells was 2,669 cells/mm² in llamas and 2,275 cells/mm² in alpacas. Density of endothelial cells decreased with age in llamas. Polymegathism was observed frequently in both species. Mean corneal thickness was 608 μm for llamas and 595 μm for alpacas. Corneal thickness and density of endothelial cells were negatively correlated in llamas. Older (>36 months old) llamas had significantly larger horizontal and vertical corneal diameters than younger llamas, and older alpacas had a significantly larger vertical corneal diameter than younger alpacas.

Conclusions and Clinical Relevance—Density of corneal endothelial cells is only slightly lower in camelids than other domestic species. Density of endothelial cells decreases with age in llamas. Age or sex does not significantly affect corneal thickness in normal eyes of llamas and alpacas. Specular microscopy is useful for determining density of corneal endothelial cells in normal eyes of camelids.

Llamas (Lama glama) and alpacas (Lama pacos) are popular pet, show, and production animals in the United States. Corneal disease is relatively frequently identified in llamas. In a retrospective study, it was determined that ocular disease happens significantly more frequently in llamas than in horses or cattle and that the cornea is the most commonly affected ocular structure in llamas with ocular disease. The authors of that report theorized that this prevalence may have been attributable to the large size and anatomic positioning of the globe, predisposing the eyes of llamas to trauma.

In that same retrospective study, cataracts were the most common lens abnormality recorded in llamas, affecting 20 of 194 (10.3%) animals examined to determine the cause of an ocular problem. Cataracts also have been reported in alpacas. In a survey of 29 alpacas, 3 had cataracts. A guanaco (Lama guanacoe) reportedly had bilateral corneal edema and unilateral cataract and lens coloboma. Veterinary ophthalmologists are hesitant to perform cataract surgery on camelids because of a propensity for the formation of severe corneal edema after surgery. The only report of cataract removal in a camelid describes a llama cria that underwent extracapsular cataract extraction and developed severe corneal edema in both eyes and an ulcer and perforation of 1 eye.

The aqueous humor of llamas and alpacas is similar to that of other species (eg, dogs, horses) that can successfully undergo cataract surgery. Because protein concentrations, pH, osmolality, and ionic contents of the aqueous humor of camelids do not differ significantly from those of other species, it was hypothesized that camelids may have a fragile corneal endothelium and that damage to it may be responsible for postoperative corneal edema.

Corneal endothelium is a single layer of hexagonally shaped cells that forms a barrier between the corneal stroma and aqueous humor. A minimum critical endothelial cell density (ECD) is necessary to maintain corneal function in humans. Corneal endothelial cells and corneal clarity may be compromised by disease processes or by surgical manipulation of the cornea or anterior chamber of the eyes.

Values for ECD have been reported in many species. Ocular pathologic and age-related changes that affect ECD have been reported in humans and dogs. To the authors’ knowledge, there have not been any reports on ECD in camelids. The objectives of the study reported here were to determine central corneal ECD, corneal thickness (CT) in 3 areas, and horizontal and vertical corneal diameters (CD) in normal eyes of llamas and alpacas. We also intended to determine whether there was an interaction of response variables (age, sex, and side [left eye vs right eye]) on ECD, CT, or CD.

Materials and Methods

Animals—Thirty-six llamas and 20 alpacas were used in
the study. Llamas ranged from 9 to 200 months old (median, 17 months). Eleven were sexually intact males (range, 3 to 47 months old; median, 10 months), 10 were castrated males (range, 11 to 20 months old; median, 15 months), and 15 were females (range, 9 to 200 months old; median, 80 months). Alpacas ranged from 22 to 204 months old (median, 48 months). Ten were sexually intact males (range, 22 to 141 months old; median, 33 months), and 10 were females (range, 27 to 204 months old; median, 48 months).

All animals were housed at a university research farm. Each camelid was manually restrained, and the anterior segment of both eyes was examined with diffuse illumination, using a transilluminator. Results of these examinations were normal. Pupil dilation was accomplished by use of a solution of 1% tropicamide. Examination of the posterior segment of each eye was performed by use of a 20-diopter indirect lens, and results were considered normal. None of the camelids had a history of ocular problems or clinical signs consistent with ocular disease.

**Corneal diameter**—Topical anesthetic (0.5% proparacaine) was applied to each eye, and Jameson calipers then were used to measure the diameter of each cornea. Diameter from limbus to limbus was measured in horizontal and vertical directions.

**Density of corneal endothelial cells**—Animals were sedated by IV administration of xylazine hydrochloride. Llamas were administered xylazine at a dose of 0.3 mg/kg, and alpacas were administered the drug at a dose of 0.6 mg/kg. Animals were positioned in sternal recumbency, and their heads were maintained in an elevated position and placed on a headrest. The ECD was determined for each eye, using a noncontact specular microscope in automatic or manual mode. The instrument used differential focusing on the corneal epithelial and endothelial cell surfaces to image the endothelial cells in selected areas. A required working distance of 25 mm was used. A photographic area of the central cornea measuring 0.2 × 0.5 mm was analyzed. Fifteen representative endothelial cells were selected for analysis. Values for minimum, maximum, and mean ± SD cell sizes were calculated.

**Corneal thickness**—Ultrasonographic pachymetry was used to determine CT at 5 locations (central, dorsal, ventral, medial, temporal) in both eyes of each animal. Corneal measurements at peripheral locations (ie, dorsal, ventral, medial, and temporal) were obtained approximately 5 mm from the limbus in the clear portion of the cornea. Corneal thickness was determined, using a 20-MHz ultrasonographic pachymeter that obtains consecutive A-scan measurements of CT during recording and internally eliminates measurements > 5 μm from the mean before displaying a CT value. The transducer was apposed gently to the cornea in each of the 5 measurement locations without indenting the cornea. A value of 1,640 m/s was used for velocity of sound through aqueous humor. The ECD was determined for each eye, and Jameson calipers then were used to measure the diameter of each cornea. Diameter from limbus to limbus was measured in horizontal and vertical directions.

**Statistical analysis**—Response variables in the study reported here were ECD, CT, horizontal CD, and vertical CD. Univariate and multivariate ANOVA were used to evaluate the effect of sex, side (right eye vs left eye), and age on these variables in llamas and alpacas. Data were expressed as mean ± SEM. Values of P < 0.05 were considered significant.

**Results**

**Density of corneal endothelial cells of llamas**—Mean central ECD for all eyes of llamas was 2,669 ± 56 cells/mm². Values did not differ significantly between right and left eyes of the same llama. The ECD decreased significantly (P = 0.004) with increasing age in llamas, but sex did not significantly affect ECD. Mean ECD in younger (≤ 36 months old) llamas was 2,778 ± 82 cells/mm², whereas mean ECD in older (> 36 months old) llamas was 2,516 ± 63 cells/mm² (Table 1). Heterogeneity of cells (polymegathism and pleomorphism) was observed frequently (Fig 1).

**Density of corneal endothelial cells of alpacas**—Mean central ECD for all eyes of alpacas was 2,275 ± 90 cells/mm². Values for ECD did not differ significantly between right and left eyes of the same alpaca. There was not a significant (P = 0.066) effect of age on ECD in alpacas, and sex did not significantly affect ECD (Table 1). Heterogeneity of cells was observed frequently.

**Corneal thickness of llamas**—Mean CT for all eyes of llamas was 608 ± 5 μm. Corneas of younger llamas were significantly (P = 0.04) thinner (592 ± 10 μm), compared with corneas for older llamas (630 ± 82 μm). Corneas of younger llamas were significantly (P = 0.04) thinner (592 ± 10 μm), compared with corneas for older llamas (630 ± 82 μm). Heterogeneity of cells (polymegathism and pleomorphism) was observed frequently (Fig 1).

**Corneal thickness of alpacas**—Mean central CT for all eyes of alpacas was 321 ± 13 μm. ECD had a history of ocular problems or clinical signs consistent with ocular disease.

**Table 1—Comparison of corneal values between older and younger camelids**

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (mo)</th>
<th>ECD (cells/mm²)</th>
<th>CT (μm)</th>
<th>HCD (mm)</th>
<th>VCD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llama</td>
<td>≤ 36</td>
<td>2,778 ± 82</td>
<td>592 ± 10</td>
<td>28.7 ± 0.2</td>
<td>21.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>&gt; 36</td>
<td>2,516 ± 63</td>
<td>630 ± 12</td>
<td>32.1 ± 0.3</td>
<td>24.2 ± 0.4</td>
</tr>
<tr>
<td>Alpaca</td>
<td>≤ 36</td>
<td>2,472 ± 122</td>
<td>590 ± 4</td>
<td>30.1 ± 0.5</td>
<td>21.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>&gt; 36</td>
<td>2,144 ± 120</td>
<td>598 ± 6</td>
<td>30.3 ± 0.2</td>
<td>22.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values reported are mean ± SEM. *For each species, values within a column with different superscript letters differ significantly (P < 0.05). ECD = Endothelial cell density. CT = Corneal thickness. HCD = Horizontal corneal diameter. VCD = Vertical corneal diameter.
12 µm; Table 1). Although it appeared that sex significantly affected CT of llamas (young males had thinner corneas than old females), there was an overrepresentation of young males and too few young females for accurate comparisons of age-sex interactions.

**Discussion**

Ocular disease is relatively common in camelids. In a comprehensive retrospective study of ocular disease in llamas, 6% of llamas examined at veterinary teaching hospitals had an ocular problem, with the cornea being the most frequently affected ocular structure (41%). Camelids have extremely large prominent corneas being the most frequently affected ocular structure.4 The corneal endothelium is vital to maintaining corneal clarity. During normal aging, cells are lost, and remaining cells spread to fill gaps. Corneal endothelium is a monolayer cell population that does not undergo mitosis.22 When there is damage to the endothelium, remaining cells enlarge to fill gaps.23 Human beings are born with approximately 1 million corneal endothelial cells, and this number decreases by two thirds throughout life.24 A minimum critical ECD of 400 to 700 cells/mm² is required to sustain corneal viability.25

Mean ECD decreased with increasing age in llamas but not alpacas in the study reported here. Humans lose approximately 0.36% of their endothelial cells annually.26 A correlation between decreasing ECD with increasing age also has been reported in rabbits,47 cats,10,b dogs,19 and horses.12 We were unable to determine the reason that the ECD in alpacas did not decrease significantly with increasing age. However, a larger sample population that would include younger animals may have revealed that increasing age is an important factor for decreases in ECD.

**Corneal thickness of llamas—Mean horizontal CD for all eyes of llamas was 28.2 ± 0.4 mm, whereas mean vertical CD was 22.5 ± 0.3 mm. Older llamas (n = 15) had significantly (P < 0.001) larger horizontal (32.1 ± 0.3 mm) and vertical CD (24.2 ± 0.4 mm), compared with corresponding values for 21 younger llamas (28.7 ± 0.2 and 21.5 ± 0.2 mm for horizontal and vertical CD, respectively; Table 1).

**Corneal thickness of alpacas—Mean horizontal CD for all eyes of alpacas was 30.2 ± 0.2 mm, whereas mean vertical CD was 22.2 ± 0.2 mm. Values for both CD did not differ significantly between the right and left eyes of the same alpaca. Older alpacas (n = 10) had significantly (P < 0.001) larger vertical CD (22.7 ± 0.2 mm), compared with the value for 10 younger alpacas (21.6 ± 0.3 mm; Table 1).**

The corneal endothelium is vital to maintaining corneal clarity. During normal aging, cells are lost, and remaining cells spread to fill gaps. Corneal endothelium is a monolayer cell population that does not undergo mitosis.22 When there is damage to the endothelium, remaining cells enlarge to fill gaps.23 Human beings are born with approximately 1 million corneal endothelial cells, and this number decreases by two thirds throughout life.24 A minimum critical ECD of 400 to 700 cells/mm² is required to sustain corneal viability.25

In the study reported here, mean ECD was 2,673 cells/mm² in llamas and 2,275 cells/mm² in alpacas. These values are only slightly lower than those reported for normal eyes of dogs (2,816 cells/mm²),77 cats (2,668 and 2,794 cells/mm²),10,b dogs (3,155 cells/mm²),10,a,b and horses (3,155 cells/mm²).10,h Heterogeneity of endothelial cells was evident in llamas and alpacas of various ages. Polymegathism (ie, variability in cell size) and pleomorphism (ie, detection of cells that are not hexagonal) may indicate stress to the endothelial layer or an abnormal cytoskeleton of the endothelial cells.26

Veterinary ophthalmologists are hesitant to remove cataracts or perform other intraocular surgery on camels because of a propensity for postoperative complications.4 Cataract removal reportedly has limited success because of severe postoperative corneal edema and uveitis.4 The edema can result in corneal swelling and ulcers that may be progressive and blind the animal.4 Many cataracts in llamas are congenital,7 and it is presumed that cataract removal is most often performed on young llamas with the highest possible cell density. There are several theories regarding the mechanism of this postoperative corneal edema. The ECD in llamas may be lower than expected, the endothelium may be extremely sensitive, or there may be severe postoperative inflammation.4,6 Results of the study reported here did not support a low ECD value as the sole cause of postoperative corneal decompensation. Polymegathism and pleomorphism of endothelial cells may mean that evaluation of ECD alone is not sufficient for assessment of endothelial stability.25 The slightly lower ECD values of camelids combined with an inherently unstable endothelial cell layer, concurrent intraocular surgery, and inflammation may be sufficient to compromise the pumping function of the endothelium. The effect of phacoemulsification or various irrigating solutions in the anterior chamber on ECD in dogs has been reported28,29; however, to our knowledge, this information has not been reported in camelids and could be another potential source of complications.

Mean ECD decreased with increasing age in llamas but not alpacas in the study reported here. Humans lose approximately 0.36% of their endothelial cells annually.26 A correlation between decreasing ECD with increasing age also has been reported in rabbits,47 cats,10,b dogs,19 and horses.12 We were unable to determine the reason that the ECD in alpacas did not decrease significantly with increasing age. However, a larger sample population that would include younger animals may have revealed that increasing age is an important factor for decreases in ECD.

**Corneal thickness of alpacas—Mean horizontal CD for all eyes of alpacas was 30.2 ± 0.2 mm, whereas mean vertical CD was 22.2 ± 0.2 mm. Values for both CD did not differ significantly between the right and left eyes of the same alpaca. Older alpacas (n = 10) had significantly (P < 0.001) larger vertical CD (22.7 ± 0.2 mm), compared with the value for 10 younger alpacas (21.6 ± 0.3 mm; Table 1).**

**Corneal thickness of camels—Mean horizontal CD for all eyes of camels was 30.2 ± 0.2 mm, whereas mean vertical CD was 22.2 ± 0.2 mm. Values for both CD did not differ significantly between the right and left eyes of the same camel. Older camels (n = 10) had significantly (P < 0.001) larger vertical CD (22.7 ± 0.2 mm), compared with the value for 10 younger camels (21.6 ± 0.3 mm; Table 1).**

**Table 1. Mean values for central corneal thickness (µm) and corneal endothelial cell density (ECD) in camelids.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Hor CD (µm)</th>
<th>Mean Vert CD (µm)</th>
<th>Mean ECD (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llama</td>
<td>28.2 ± 0.4</td>
<td>22.5 ± 0.3</td>
<td>2,673 ± 0.2</td>
</tr>
<tr>
<td>Alpaca</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
<tr>
<td>Camel</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
</tbody>
</table>

**Table 2. Mean values for horizontal and vertical CD (µm) and ECD (cells/mm²) in camelids.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Hor CD (µm)</th>
<th>Mean Vert CD (µm)</th>
<th>Mean ECD (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llama</td>
<td>28.2 ± 0.4</td>
<td>22.5 ± 0.3</td>
<td>2,673 ± 0.2</td>
</tr>
<tr>
<td>Alpaca</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
<tr>
<td>Camel</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
</tbody>
</table>

**Table 3. Mean values for horizontal and vertical CD (µm) and ECD (cells/mm²) in camelids.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Hor CD (µm)</th>
<th>Mean Vert CD (µm)</th>
<th>Mean ECD (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llama</td>
<td>28.2 ± 0.4</td>
<td>22.5 ± 0.3</td>
<td>2,673 ± 0.2</td>
</tr>
<tr>
<td>Alpaca</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
<tr>
<td>Camel</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
</tbody>
</table>

**Table 4. Mean values for horizontal and vertical CD (µm) and ECD (cells/mm²) in camelids.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Hor CD (µm)</th>
<th>Mean Vert CD (µm)</th>
<th>Mean ECD (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llama</td>
<td>28.2 ± 0.4</td>
<td>22.5 ± 0.3</td>
<td>2,673 ± 0.2</td>
</tr>
<tr>
<td>Alpaca</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
<tr>
<td>Camel</td>
<td>30.2 ± 0.2</td>
<td>22.2 ± 0.2</td>
<td>2,275 ± 0.2</td>
</tr>
</tbody>
</table>

3. Tropicamide, Bausch & Lomb Pharmaceutical, Tampa, Fla.
4. Afalaine, Alcon Laboratories Inc, Fort Worth, Tex.
5. Rompun 30 mg/ml, Bayer Corp, Shawnee Mission, Kan.
6. Topcon SP-2000P Topcon America, Paramus, NJ.
7. DGH500, DGH Technology Inc, Exton, Pa.

References


