Results of diagnostic ophthalmic testing in healthy guinea pigs

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Objective—To report values for tear production, central corneal touch threshold (CTT), and intraocular pressure (IOP) in healthy guinea pigs and determine results of aerobic bacterial culture and cytologic examination of conjunctival swab specimens.

Design—Cross-sectional study.

Animals—31 healthy guinea pigs (62 eyes) of various ages and breeds.

Procedures—Tear production was measured by the phenol red thread tear test (PRT) and Schirmer tear test (STT) before and after topical anesthetic application, CTT was measured with an esthesiometer, and IOP was measured by applanation tonometry.

Results—Combining data from all eyes, mean ± SD PRT values before and after topical anesthetic administration were 21.26 ± 4.19 mm/15 s and 22.47 ± 3.31 mm/15 s, respectively, and mean IOP was 18.27 ± 4.55 mm Hg. Median STT values before and after topical anesthetic administration were 3 mm/min (range, 0 to 12 mm/min) and 4 mm/min (range, 0 to 11 mm/min), respectively, and median CTT was 2.0 cm (range, 0.5 to 3.0 cm). Values did not differ between eyes for any test, but significant differences were identified for PRT values between males and females and between values obtained before and after topical anesthetic administration. Common bacterial isolates included Corynebacterium spp, Streptococcus spp, and Staphylococcus spp. Cytologic examination of conjunctival swab specimens revealed mainly basal epithelial cells; lymphocytes were common.

Conclusions and Clinical Relevance—Results provided information on values for PRT, STT, CTT, and IOP in healthy guinea pigs and on expected findings for aerobic bacterial culture and cytologic examination of conjunctival swab specimens. (J Am Vet Med Assoc 2008;232:1825–1833)

Over the past several years, guinea pigs (Cavia porcellus) have become increasingly popular as domestic pets and laboratory animals.1 Given this and the high prevalence of ocular disease in this species,2 there is a need to evaluate methods for ophthalmic testing and ocular disease recognition. Although a previous study3 reported results of the PRT and STT and values for corneal sensitivity in guinea pigs, all guinea pigs were of the Duncan-Hartley laboratory strain and of similar age. In addition, that study did not determine whether PRT values were affected by use of topical anesthetic. The purpose of the study reported here, therefore, was to report values for the PRT, STT, CTT, and IOP in healthy guinea pigs of various ages and breeds and determine results of aerobic bacterial culture and cytologic examination of conjunctival swab specimens.

Materials and Methods

Animals—Thirty-one healthy guinea pigs examined as outpatients at the Purdue University Veterinary Teaching Hospital between December 2006 and March 2007 or owned by individuals affiliated with the teaching hospital were included in the study. The study was performed in compliance with Purdue University guidelines for research. Owners of all guinea pigs used in the study provided their consent prior to study enrollment.

Study protocol—For all animals included in the study, information on age, sex, neuter status, body weight, and predominant breed was obtained. In addition, information on usual diet and environment and any previous systemic or ocular health problems was obtained when possible. A brief physical examination was performed on all guinea pigs, and animals with evidence of ocular or systemic disease, including nasal or ocular discharge, generalized alopecia, rough hair coat, or signs of wasting, were excluded.

In all animals, a complete ophthalmic examination, including assessment of menace responses and dazzle and pupillary light reflexes, was performed. All eyes were evaluated by means of slit-lamp biomicroscopy and indi-

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
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<tbody>
<tr>
<td>CTT</td>
<td>Corneal touch threshold</td>
</tr>
<tr>
<td>IOP</td>
<td>Intraocular pressure</td>
</tr>
<tr>
<td>PAS</td>
<td>Periodic acid–Schiff</td>
</tr>
<tr>
<td>PRT</td>
<td>Phenol red thread tear test</td>
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<tr>
<td>STT</td>
<td>Schirmer tear test</td>
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</table>

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The Shapiro-Wilk test was not necessary because of incomplete pupillary constriction. For all subsequent examinations, the first eye tested was alternated between subjects.

Following completion of the ophthalmic examination, the PRT and STT were performed. Ten minutes was allowed to elapse between tests to allow time for the tear film to restabilize.4 The order of tear testing was switched after every 2 animals, so that the PRT was performed first for half of the study population and the STT was performed first for the other half, with an equal distribution of left or right eyes being tested first for each test.

For the PRT, test threads from a single lot6 were used in all animals. The lower eyelid was pulled down, and the 3-mm folded end of the thread was placed in the inferior conjunctival fornix. After 15 seconds, the thread was removed and the entire wetted portion of the thread, regardless of whether it had turned red, was measured from the thread tip with the measuring scale that had been enclosed with the test threads.

For the STT, test strips from a single lot6 were used in all animals. The test strip was folded at the notch and placed in the inferior conjunctival fornix. The eyelids were held shut for 1 minute, and the length of the wetted portion of the strip was determined by comparison with the scale imprinted on the strip. A value of 0 mm/min was recorded for eyes in which wetting did not reach the notch on the strip. A value of 1 mm/min was recorded for eyes in which wetting stopped at the notch on the strip.

Swab specimens were then obtained from each eye for aerobic bacterial culture. For collection of swab specimens, a moistened sterile cotton-tipped swab7 was placed in the inferior conjunctival fornix and rotated, as described.8 Swab specimens were refrigerated at 4°C after collection and submitted within 4 hours for standard aerobic culture. For all isolates, susceptibility to commonly used ophthalmic antimicrobials was determined by means of microbroth dilution.

The CTT was determined as described7–8 with an aesthesiometer6 equipped with a 0.12-mm-diameter nylon filament. For each eye, filament length was initially set at 4.0 cm. The filament was touched perpendicularly to the central axial portion of the cornea until slight flexion of the filament was evident, and the eye was observed for blinking. If a consistent blink was not elicited, the filament length was decreased by 0.5 cm for each subsequent reading. When a blink was elicited with at least 3 of 5 stimulations performed with a particular filament length, that length was recorded as the CTT. If no response was observed at a filament length of 0.5 cm, CTT was recorded as 0 cm. After statistical analysis, filament length was converted into a pressure measurement (g/mm²) by use of the manufacturer's conversion table.

A single drop of 0.5% proparacaine hydrochloride was then instilled in each eye to induce corneal anesthesia. After approximately 20 seconds, the inferior conjunctival fornix was dried with cotton.4 Bottles of proparacaine designated for this study were refrigerated at 4°C to maintain consistent efficacy between animals and were discarded within 6 weeks after they were opened.9

Following instillation of proparacaine, conjunctival swab specimens were obtained with an applicator brush10 for cytologic examination. The brush was rotated in the inferior conjunctival fornix in a single direction 5 times and then lightly rolled onto a clean glass slide, as described.11,12 Slides were air-dried and then stained with a modified Wright stain. Slides were examined by means of light microscopy at 40× magnification to identify areas of high cellularity with well-preserved cells. Differential cell counts were then performed at 400× magnification. For each slide, 200 nucleated cells from 3 randomly selected locations were counted, for a total of 600 cells. Multilayered and ruptured cells were ignored. Epithelial cells were classified on the basis of descriptions of conjunctival cells in domestic species.11,12 Slides from 8 animals (16 eyes) were stained with alcian blue stain with the PAS reaction and examined to assess the pattern of staining for acidic (blue), neutral (pink), and mixed (purple) mucopolysaccharide components.

Intraocular pressure was subsequently measured by means of applanation tonometry in accordance with the manufacturer's directions. Because diurnal variation is known to alter IOP in other species,13,14 the time of day that IOP was measured was recorded. Toward the end of the study, a rebound tonometer5 became available, and IOP was measured by means of rebound tonometry immediately after applanation tonometry in 5 animals (10 eyes). Rebound tonometry was performed in accordance with the manufacturer's directions; the “P” calibration setting was used for all measurements.

Finally, the PRT and STT were repeated in all eyes, with 10 minutes allowed to elapse between tests. The order of testing was the same as the order prior to instillation of proparacaine. In addition, CTT was measured immediately before each test. If the CTT was not 0, an additional drop of proparacaine was administered to both eyes, the conjunctival fornix was dried, and 10 minutes was allowed for the tear film to stabilize before testing was performed.

For all ophthalmic testing, animals were gently physically restrained. Testing was delayed or discontinued in any animal that could not be restrained or became excessively vocal. Therefore, data sets were incomplete for some animals. All examinations were performed by a single individual (MEC).

Three months after the conclusion of the study, one of the guinea pigs was found dead in its cage and was brought to Purdue University for necropsy. Both globes were processed routinely for histologic examination. Individual slides were stained with both H&E and alcian blue with PAS reaction and hematoxylin counterstain.

**Comparison population**—To determine whether the study population was representative of the population of guinea pigs typically examined at Purdue University, medical records of a comparison population consisting of all guinea pigs examined at the Purdue University Veterinary Teaching Hospital for primary care between January 2001 and December 2006 were examined, and information on age, sex, neuter status, and final diagnoses was obtained.

**Statistical analysis**—The Shapiro-Wilk test was used to determine whether data were normally dis-
distributed. To determine whether the sample population was representative of guinea pigs examined at Purdue University, age, sex, and neuter status were compared between the sample and comparison populations by use of the Mann-Whitney U, \( \chi^2 \), and Fisher exact tests, respectively. For animals in the sample population, the Spearman rank correlation method was used to test for a correlation between age and weight; data for males and females were examined separately.

For continuous normally distributed data, mean and SD were calculated for all eyes combined and for right and left eyes separately. For skewed data, the median and range of values were reported and nonparametric testing was performed.

Data for PRT and IOP were analyzed by repeated-measures ANOVA, with eye (left or right) and, for PRT data, anesthetic state included as repeated measures. Age, sex, weight, predominant breed (Abyssinian, American, or other) and, for IOP data, time of day of measurement were assessed as independent effects. Neither STT nor CTT data were normally distributed. The Friedman ANOVA was therefore used to assess the effect of eye and anesthetic state on STT data. The Wilcoxon signed rank test was used to assess the effect of eye on CTT data.

The Spearman rank correlation method was used to compare PRT and STT values obtained before application of proparacaine with values obtained after application of proparacaine; data for the left and right eyes were analyzed separately. Paired \( t \) tests were used to compare IOP values obtained by applanation tonometry with values obtained by rebound tonometry; again, values for the left and right eyes were analyzed separately. The Bland-Altman method was used to assess the level of agreement between IOP values obtained with the 2 methods; values for the left and right eyes were analyzed separately.

Standard software was used for all analyses. Values of \( P < 0.05 \) were considered significant.

**Results**

**Animals**—Thirty-one healthy guinea pigs were enrolled in the study. There were 16 males and 15 females. Three additional animals were excluded from the study; one had bilateral incipient cataracts, one had an abdominal mass and generalized alopecia, and one had eosinophilic conjunctivitis. Data sets were incomplete for 2 of the animals included in the study; STT could not be measured after instillation of proparacaine in a male guinea pig, and IOP and both PRT and STT could not be measured after instillation of proparacaine in a female guinea pig because of overt signs of stress.

All guinea pigs included in the study appeared to be in good health. Response to menace was weak in 3 of 31 (10%) guinea pigs and absent in all others. Only 4 of 31 (13%) guinea pigs had a dazzle reflex when examined with a halogen transilluminator. Direct and consensual pupillary light reflexes were slow and incomplete in all animals. No abnormalities were noticed during slit-lamp biomicroscopy or indirect ophthalmoscopy in any animal.

Median age was 12 months (range, 1.5 to 42 months). Mean ± SD body weight was 0.96 ± 0.26 kg (2.1 ± 0.57 lb). Age and weight were positively correlated in males \( (r_s = 0.85; P < 0.01) \) and females \( (r_s = 0.68; P < 0.01) \). There were 22 Abyssinian guinea pigs, 7 American guinea pigs, 1 White Crested guinea pig, and 1 Duncan-Hartley guinea pig.

All guinea pigs were fed a diet consisting of grass hay, pelleted food containing stabilized vitamin C, and fresh leafy vegetables, and were kept in cages. Information regarding additional vitamin C supplementation was not obtained. To the owners’ knowledge, none of the animals had previously had any ophthalmic diseases or been treated with antimicrobials.

**Comparison population**—Analysis of medical records revealed that 82 guinea pigs were examined at the Purdue University Veterinary Teaching Hospital for

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of eyes</th>
<th>No. of guinea pigs</th>
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<tbody>
<tr>
<td>Gram-positive bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corynebacterium spp</td>
<td>47</td>
<td>27</td>
</tr>
<tr>
<td>( \alpha )-hemolytic Streptococcus spp</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Micrococcus spp</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Aerococcus spp</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Bacillus spp</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Myxoides spp</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pantoea agglomerans</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No growth</td>
<td>7</td>
<td>6</td>
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</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of guinea pigs</th>
<th>No. of eyes</th>
<th>Mean ± SD or median</th>
<th>Range</th>
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<tbody>
<tr>
<td>PRT (mm/15 s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before topical anesthesia</td>
<td>31</td>
<td>62</td>
<td>21.26 ± 4.19</td>
<td>11–32</td>
</tr>
<tr>
<td>After topical anesthesia</td>
<td>30</td>
<td>60</td>
<td>22.47 ± 3.31</td>
<td>16–30</td>
</tr>
<tr>
<td>STT (mm/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before topical anesthesia</td>
<td>31</td>
<td>62</td>
<td>3</td>
<td>0–12</td>
</tr>
<tr>
<td>After topical anesthesia</td>
<td>29</td>
<td>58</td>
<td>4</td>
<td>0–11</td>
</tr>
<tr>
<td>CTT (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals</td>
<td>31</td>
<td>60</td>
<td>18.27 ± 4.55</td>
<td>8–28</td>
</tr>
</tbody>
</table>

*Measured by means of applanation tonometry.
primary care between January 2001 and December 2006. Of these, 36 were male and 23 were female (sex of 1 animal was not recorded). Information on neuter status was available for only 21 of the 82 animals; 11 were sexually intact and 10 were neutered. Median estimated age at the time of initial examination at the veterinary teaching hospital was 12.8 months (range, 0.7 to 62 months). Fourteen of the animals were healthy at the time of examination. Six animals had ocular disease, including 2 animals with ocular discharge related to upper respiratory tract infection and 1 animal each with conjunctivitis, dacryocystitis, corneal scarring, and an orbital foreign body. Other reasons for examination of guinea pigs included respiratory tract disease (9), gastrointestinal tract disease (3), neurologic examination of conjunctival swab specimens obtained from the right and left eyes of 31 healthy guinea pigs. Error bars represent SD. Figure 1—Mean percentages of various types of epithelial cells seen during cytologic examination of conjunctival swab specimens obtained from the right and left eyes of 31 healthy guinea pigs. Error bars represent SD.

### Table 3—Results of antimicrobial susceptibility testing of 57 bacterial isolates obtained from conjunctival swab specimens in healthy guinea pigs.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th><em>Corynebacterium</em> spp (n = 26)</th>
<th><em>α</em>-hemolytic <em>Streptococcus</em> spp (n = 11)</th>
<th><em>Staphylococcus epidermidis</em> (n = 14)</th>
<th>Other gram-positive isolates (n = 10)</th>
<th>Gram-negative isolates (n = 13)</th>
<th>All isolates (n = 57)</th>
<th>Percentage of isolates resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Neomycin</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Oxacillin P&lt; sub&gt;8&lt;/sub&gt;</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>1</td>
<td>5</td>
<td>12</td>
<td>51</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>2</td>
<td>3</td>
<td>13</td>
<td>5</td>
<td>12</td>
<td>35</td>
<td>61</td>
</tr>
</tbody>
</table>

Age and sex distributions were not significantly different between the sample and comparison populations. However, guinea pigs in the sample population were significantly (P < 0.01) more likely to be sexually intact than were guinea pigs in the comparison population. Even after exclusion of guinea pigs in the comparison population that were examined primarily for neutering (and had been included in the data set as neutered), guinea pigs in the sample population were significantly (P = 0.03) more likely to be sexually intact than were guinea pigs in the comparison population.

### Ophthalmic diagnostic tests

Results of ophthalmic diagnostic testing were summarized (Table 1). Mean ± SD PRT value before instillation of proparacaine was 19.88 ± 3.71 mm/15 s in males (right eyes, 19.13 ± 4.05 mm/15 s; left eyes, 20.63 ± 3.30 mm/15 s) and 22.73 ± 4.32 mm/15 s (right eyes, 22.00 ± 4.78 mm/15 s; left eyes, 23.47 ± 3.58 mm/15 s) in females. After instillation of proparacaine, mean PRT value was 21.66 ± 2.80 mm/15 s (right eyes, 22.00 ± 2.37 mm/15 s; left eyes, 21.31 ± 3.22 mm/15 s) in males and 23.39 ± 3.63 mm/15 s (right eyes, 23.50 ± 4.38 mm/15 s; left eyes, 23.29 ± 2.87 mm/15 s) in females.

Males had significantly (P < 0.01) lower PRT values than did females, and the PRT value was significantly (P < 0.05) lower before instillation of proparacaine than after. Values for the PRT were not significantly (P > 0.05) affected by eye, age, weight, or breed.

For both the left and right eyes, median STT value before instillation of proparacaine was 3 mm/min (range for right eyes, 0 to 11 mm/min; range for left eyes, 0 to 12 mm/min). Median STT value after instillation of proparacaine was 3 mm/min (range, 0 to 11 mm/min) for right eyes and 4 mm/min (range, 0 to 11 mm/min) for left eyes. Values for the STT were not significantly affected by eye or anesthetic state. There were no significant correlations between PRT and STT values obtained before or after topical anesthesia in either eye.

Median value for the CTT was 2.0 cm for the right and left eyes (range for right eyes, 0.5 to 3.0 cm; range for left eyes, 1.0 to 3.0 cm). This equated to a median pressure of 6.64 g/mm² (range for right eyes, 3.20 to 17.68 g/mm²; range for left eyes, 3.20 to 12.84 g/mm²). Ten minutes after instillation of proparacaine, corneal sensation was present in 5 eyes of 3 guinea pigs.
20 minutes, a variable degree of corneal sensation had returned to all eyes. Values for the CTT were not significantly affected by eye.

Mean ± SD IOP obtained by means of applanation tonometry was 18.27 ± 4.55 mm Hg (right eyes, 17.83 ± 4.56; left eyes, 18.70 ± 4.58). Intraocular pressure was not significantly affected by eye, age, weight, breed, sex, or time of day. For the 5 guinea pigs (10 eyes) in which IOP was measured by means of applanation and rebound tonometry, mean IOP obtained by means of applanation tonometry was 19.40 ± 2.50 mm Hg (right eyes, 18.40 ± 2.51 mm Hg; left eyes, 20.10 ± 2.30 mm Hg), and mean IOP obtained by means of rebound tonometry was 6.10 ± 2.18 mm Hg (right eyes, 5.80 ± 2.59 mm Hg; left eyes, 6.40 ± 1.94 mm Hg). Values obtained by means of the 2 tonometry methods were significantly (P < 0.001) different, and Bland-Altman analysis revealed a high level of disagreement between the 2 methods. Rebound tonometry gave IOP values 8 to 17 mm Hg (right eyes) and 8 to 20 mm Hg (left eyes) lower than values obtained by means of applanation tonometry (95% limits of agreement). Subjectively, rebound tonometry was quicker and easier to perform.

Conjunctival microflora—For 30 guinea pigs (55 eyes), aerobic bacterial culture of conjunctival swab specimens yielded bacterial growth. The most common isolates were *Corynebacterium* spp, α-hemolytic *Streptococcus* spp, and *Staphylococcus epidermidis* (Table 2). Antimicrobial susceptibility testing was performed on 57 of the 134 (43%) isolates (Table 3). Resistance to antimicrobials commonly used for topical treatment was evident.

Cytologic findings—Mean ± SD percentages of conjunctival cells identified as epithelial cells were 66 ± 21% for swab specimens from the right eyes and 63 ± 23% for specimens from the left eyes, with leukocytes accounting for the remainder of the cells. Erythrocytes were noticed in swab specimens from 17 of the 31 (55%) right eyes and 18 of the 31 (58%) left eyes. Basal, intermediate, and columnar cells accounted for most of the epithelial cells observed (Figure 1). Basal cells had a high nuclear-to-cytoplasmic ratio, with blue to dark-blue cytoplasm. Intermediate cells had a low nuclear-to-cytoplasmic ratio, with pale-blue cytoplasm. Columnar cells had a basal nucleus with microvilli at the apical side. Superficial cells were flattened and polyhedral and had a very low nuclear-to-cytoplasmic ratio with light pink-blue cytoplasm; these were rare (Figure 2). Inclusion bodies were not observed in any specimen. A few epithelial cells contained fine perinuclear intracytoplasmic vacuoles. These vacuolated epithelial cells, distinct from goblet cells, were recorded as basal, intermediate, or columnar epithelial cells on the basis of their predominant morphology (Figure 3). Slides stained with alcin blue and PAS stains revealed low numbers of cells with mucopolysaccharide components; these cells were consistent with the quantity and conformation of goblet cells.

Lymphocytes were present in variable numbers in all eyes and accounted for most of the leukocytes observed (Figure 4). Small- to medium-sized lymphocytes were most common.

Results of histologic examination of conjunctival epithelium from the guinea pig that underwent necropsy were similar to findings expected for other species.12,15 Lymphoid tissue was present underlying stratified cuboidal epithelium in the zone of the fornix. Goblet cells were rarely seen, and alcin blue staining with the PAS reaction revealed goblet cells only in the zone of the bulbar conjunctiva.
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Discussion

Results of the present study provided information on ranges of values for PRT, STT, CTT, and IOP in healthy guinea pigs. The similarity in age and sex distribution between the sample population and a comparison population of guinea pigs examined at Purdue University for primary care suggested that values were applicable to guinea pigs examined for primary care.

Values for the PRT and STT in the present study were higher than those previously reported for homogenous populations of Duncan-Hartley guinea pigs (mean ± SD, 16 ± 4.7 mm/15 s and 0.36 ± 1.09 mm/min, respectively). If these are true differences, they could be a result of natural biological variation or differences in population characteristics, as guinea pigs in the present study were of various ages and breeds. Values for PRT in the present study were similar to those reported for cats and rabbits (measured from the fold of the thread) but lower than those reported for dogs and horses. Although we found a significant difference between PRT values for males and females in the present study, the difference was small and likely not clinically important. The previous guinea pig study did not examine sex effects, and sex effects on PRT values have not been detected in other species.

The difference in STT values between the present study and a previous study could be related to differences in the manufacturer, design, and absorptive capabilities of the tear strips used in the 2 studies or to differences in how values were recorded for animals in which wetting did not reach to or beyond the notch in the strip. A standardized method for recording low (< 2 mm/min) STT values is needed.

The lack of correlation between PRT and STT values in the present study may have been attributable to the low sample size. However, a similar study involving 103 human patients undergoing cataract surgery found only weak agreement between results of these tests. The low agreement was hypothesized to be related to the fact that each test measured a different aspect of the tear film. In humans, the PRT likely measures the residual volume of tears in the conjunctival fornix, because it appears to cause less mechanical irritation than the STT and, therefore, stimulates less reflex tearing.

In the present study, however, the similarity between PRT and STT values obtained before and after instillation of proparacaine suggested that there is minimal reflex tearing in guinea pigs. The fact that PRT values were higher than STT values in the present study may reflect differences in the absorptive capabilities of the test threads versus the test strips.

Although we hypothesized that topical anesthesia would decrease values for PRT and STT, we did not find any difference in STT values obtained before and after topical anesthesia and found that PRT values measured after topical anesthesia were actually higher than those measured before topical anesthesia. However, results we obtained after topical anesthesia may have been affected by the repeated ocular and periorcular stimulation that occurred as diagnostic testing was performed. Importantly, erythrocytes were seen in > 50% of the cytologic preparations, suggesting that collection of swab specimens may have caused blood or serum leakage. Alternatively, the conjunctival sac may have been incompletely blotted dry after application of the topical anesthetic, leaving residual fluid in the fornix. The previous study of the STT in guinea pigs also did not find a significant difference between values obtained before and after topical anesthesia.

The range of values for CTT obtained in the present study encompasses values obtained by Strughold in 1930 (12 g/mm²), who used different techniques, and those in a recent report in which comparable techniques were used (3.7 g/mm²). Values for CTT vary between dogs, cats, and horses, and these differences may be related to differences in population characteristics as well as differences in esthesiometer handling and interpretation of blink reflexes. In Strughold’s direct comparison of corneal sensitivity in various species, the range of corneal sensitivities in guinea pigs was less (a higher pressure was required to elicit a blink) than ranges for cats, dogs, pigeons, rats, and rhesus macaques. The relatively low corneal sensitivity in guinea pigs may account for the lack of effect of topical anesthetic on tear test values. If reflex tearing contributed substantially to tear test values in guinea pigs, corneal anesthesia should result in a lower STT value. Although this study was not designed to assess recovery times of corneal sensitivity, esthesiometry performed immediately before tear tests were repeated revealed that corneal reflexes returned in a few eyes within 10 minutes and in all eyes within 20 minutes after instillation of proparacaine. This should be considered when performing procedures on guinea pig corneas under topical anesthesia.

Values for IOP obtained by means of applanation tonometry in the present study were greater than those reported previously (mean ± SD, 14.4 ± 3.5 mm Hg for
94 readings from 40 guinea pigs and 15.6 ± 0.9 mm Hg for 24 eyes of 24 guinea pigs) in studies25,26 that used a micromanometer in cannulated eyes of guinea pigs anesthetized with ketamine and xylazine.25,28 We are aware of only 1 study27 that included measurements of IOP by means of application tonometry in guinea pigs (20 eyes in 10 guinea pigs) that were not anesthetized. Baseline IOP in that study was approximately 19 mm Hg, which was similar to values obtained in the present study. Application tonometry has previously been validated in rats and mice.26,27 Concurrent measurement of IOP with a micromanometer and tonometer would be necessary to more precisely determine the accuracy of application tonometry in guinea pigs.

In rats and mice, IOPs obtained by means of rebound tonometry have been shown to correlate with IOPs obtained by means of direct cannulation.30–32 The lack of agreement between IOPs determined by means of rebound and application tonometry in the present study must be interpreted with caution because of the low sample size. However, rebound tonometry yielded much lower IOPs in all 10 eyes evaluated, and Bland–Altman analysis revealed that values obtained with the 2 methods differed to a clinically important degree. Performing application tonometry first could potentially have introduced a systematic error by altering IOP33,34 as has been reported in rats with glaucoma,35 but order of tonometry method made no difference in IOP measurements obtained in healthy rats, dogs, and horses.33,36 Additionally, in a study37 of pygmy goats, use of the “P” calibration setting on the rebound tonometer resulted in IOP values approximately 4 mm Hg lower than those obtained with the “D” setting. We chose the “P” setting because we obtained nearly identical readings with the “P” and “D” settings in the first 2 animals evaluated, but we did not test all 5 animals with both settings. Results of rebound and application tonometry correlate strongly in dogs and horses, although tonometer-specific reference ranges for each species are advocated because of significant differences between tonometers.36,38 In rats34 and rabbits,39 rebound tonometry was more accurate than application tonometry, and results were less variable. However, preliminary work in raptors has suggested that corneal thickness may affect individual tonometer readings.40 Further study with a larger sample size and incorporating direct manometry and corneal pachymetry measurements is needed to establish a reference range for IOP obtained by means of rebound tonometry in guinea pigs.

Aerobic bacterial culture of conjunctival swab specimens in the present study yielded predominantly gram-positive bacteria, with Corynebacterium spp, α-hemolytic Streptococcus spp, and S epidermidis isolated most frequently. These organisms likely represent nonpathogenic commensal flora of the conjunctiva, and similar organisms have been isolated from other species.40–48 The fourth most commonly identified organism, Micrococcus sp, is part of the normal flora in other exotic animals.3,49 Gram-negative bacteria were less commonly isolated than gram-positive bacteria. Specific culture methods for Chlamyphila spp and Bordetella spp, which are known to cause conjunctivitis in guinea pigs,50 were not employed. However, the diagnosis of Chlamyphila con-

junctivitis typically relies on cytologic results and testing with a PCR assay.51 Cytologic examination of conjunctival specimens did not yield evidence of Chlamyphila inclusions in any guinea pigs in the present study, but organisms may be only rarely found in animals with chronic conjunctivitis.32,53

Antimicrobial treatment must be carefully selected in guinea pigs to avoid antimicrobial-associated enterotoxemia seen with certain penicillins, tetracyclines, lin- cosamides, macrolides, and bacitracin.44,55 Substantial amounts of antimicrobials may be absorbed systemically following topical administration for the treatment of conjunctivitis.2,56 Antimicrobials generally considered safe for systemic use in guinea pigs include doxycycline and the fluoroquinolones.55 Thus, use of topical formulations of these compounds should be safe. In addition, use of a combination of oxytetracycline and polymyxin B sulfate is the treatment of choice for guinea pigs with chlamydophilosis.31 Infectious conjunctivitis is likely the most common primary ocular disease of guinea pigs,30,51 and knowledge of the normal flora and susceptibility profiles should aid in antimicrobial selection. Further study of the effects of topical antimicrobial administration on the ocular and gastrointestinal tract flora in guinea pigs is needed.

The differential cell count of conjunctival epithelial cells in the present study was similar to that reported for horses.12 On histologic examination of samples from a single guinea pig, goblet cells were seen only in the bulbar conjunctiva, although they have previously been reported to occur in various numbers throughout the perilimbal, bulbar, and palpebral conjunctiva of this species.37–40 It is unclear how this finding relates to the apparent low tear production of this species, and further information on the contributions of goblet cells, lacrimal glands, and meibomian glands to the tear film in guinea pigs is needed. Epithelial cells with intracytoplasmic vacuoles were observed in low numbers in the present study. These types of cells were described as mucus cells in a study12 of conjunctival specimens from horses. The morphology of these cells was more consistent with columnar, basal, and intermediate cells than with the morphology of goblet cells. However, only 45% of the columnar mucus cells and 3% of the intermediate and basal mucus cells in horses stained positively with PAS stain.12 In the 16 conjunctival specimens treated with a combination of oxytetracycline and polymyxin B sulfate in the present study, cells that stained positively with PAS stain were consistent with goblet cells in their morphology and quantity, and only goblet cells had a positive reaction in the histologic specimens. The intracytoplasmic vacuoles seen in epithelial cells, therefore, do not appear to contain glycogen or mucin. The vacuoles may have intracellular transport and autophagocytic functions,58 or these cells could be immature goblet cells.80

Percentages of conjunctival cells classified as leukocytes in the present study were higher than percentages reported in other species.10–12 Ultrastructural studies57–60 of guinea pig conjunctiva have shown lymphocytes to be present in all layers of the conjunctiva at the fornix, and lymphoid aggregates were seen in the superficial layers of connective tissue in the lamina propria at the fornix of the single animal in which the fornix was examined.
in the present study. In animals that lack any clinical evidence of conjunctivitis, lymphocytes and plasma cells are considered normal cytologic findings. Other leukocytes were consistent with blood contamination, likely as a result of disruption of small conjunctival vessels.

a. Zone-Quick, lot #61180, FCI Ophthalmics, Pembroke, Mass.
b. Schirmer tear test, lot #6090621, Schering-Plough Animal Health Corp, Union, NJ.
c. BBL CultureSwab, Becton, Dickinson & Co, Sparks, Md.
d. Cochet-Bonnet aesthesiometer, Luneau, Chartres Cedex, France.
e. Microbrush disposable applicator, regular size, Microbrush International, Graffon, Wis.
f. TonoPen-Vet, Medtronic Solan, Jacksonville, Fla.
g. TonoVet, Veclco, St Joseph, Mo.
h. SAS, version 9.1.3, SAS Institute Inc, Cary, NC.
i. SPSS, version 14.0.2, SPSS Inc, Chicago, Ill.

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