**Effects of topical administration of 0.005% latanoprost solution on eyes of clinically normal horses**

A. Michelle Willis, DVM; Kathryn A. Diehl, DVM, MS; Stacy Hoshaw-Woodard, PhD; Ichirio Kobayashi, DVM; Michael P. Vitucci, BS; L. Michael Schmall, DVM, MS

**Objective**—To determine the effect of 0.005% latanoprost solution on intraocular pressure (IOP) of eyes of clinically normal horses and establish the frequency of adverse effects of drug administration.

**Animals**—20 adult clinically normal horses.

**Procedure**—IOP was recorded (7, 9, and 11 AM; 3, 5, and 7 PM) on days 1 and 2 (baseline), days 3 to 7 (treatment), and days 8 to 9 (follow-up). Latanoprost was administered to 1 randomly assigned eye of each horse every 24 hours during the treatment period, following the 7 AM IOP recording. Pupil size and the presence or absence of conjunctival hyperemia, epiphora, blepharospasm, blepharedema, and aqueous flare were recorded prior to IOP measurement.

**Results**—IOP was reduced from baseline by a mean value of 1.03 mm Hg (5%) in males and 3.01 mm Hg (17%) in females during the treatment period. Miosis developed in all treated eyes and was moderate to marked in 77% of horses, with the peak effect observed 4 to 8 hours after drug administration. Conjunctival hyperemia, epiphora, blepharospasm, and blepharedema were present in 100, 57, 42, and 12% of treated eyes, respectively, 2 to 24 hours following drug administration. Aqueous flare was not observed at any time point.

**Conclusions and Clinical Relevance**—Although IOP was reduced with every 24-hour dosing of latanoprost, the frequency of prostaglandin-induced adverse events was high. Because recurrent uveitis appears to be a risk factor for glaucoma in horses, topical administration of latanoprost may potentiate prostaglandin-mediated inflammatory disease in affected horses. (Am J Vet Res 2001;62:1945–1951)

Glucoma is a disease resulting from an alteration in aqueous humor dynamics that causes an increase in intraocular pressure (IOP) above what is compatible with normal ocular function. Glaucoma in horses is a recognized clinical syndrome in veterinary ophthalmology, the cause of which may be congenital, primary (absence of concurrent predisposing ophthalmic disease), or secondary to intraocular neoplasia or inflammation associated with trauma or equine recurrent uveitis. Even if a preexisting inflammatory disease can be managed, established glaucoma can irreversibly damage the retina and optic nerve and can contribute to visual dysfunction.

The primary goal of glaucoma treatment in any species is to maintain IOP within a range that is compatible with intraocular health, ocular comfort, and sustained visual function. Treatment is typically aimed at increasing aqueous humor outflow from the eye or reducing aqueous humor production. Medical management is the first line of glaucoma treatment in humans and dogs and is also routinely performed to augment surgical glaucoma treatment in these species. Standard medical options for glaucoma treatment include topical and systemic administration of carbonic anhydrase inhibitors and topical administration of miotics, β-blockers, adrenergic agents, and prostaglandin (PG) analogs.

Results of clinical studies and investigations on the anatomy and aqueous humor dynamics of the eye in horses indicate that this species is likely to have different responses to the topical administration of glaucoma medications currently available, compared with the responses observed in humans and dogs. Additionally, physiologic characteristics such as corneal and conjunctival surface area, rate of blinking, and tear turnover vary among animal species and can affect drug absorption and, thus, target tissue response. Extrapolation from data on the efficacy of topical drug administration from other species to horses is therefore not recommended.

Prostaglandin analogs are a new class of ocular hypotensive drugs that have been developed for the treatment of primary open angle glaucoma in humans. Low doses of naturally occurring PG reduce IOP in other species including cats, monkeys, rabbits, and dogs. Species differences are found in terms of efficacy and mechanism of action among the different PG. Prostaglandin F2α has been extensively tested in human eyes. Although it is an effective ocular hypotensive drug, in its natural state it is not clinically useful as the result of pronounced ocular adverse effects, mainly conjunctival hyperemia and ocular irritation, at doses that induce a maximal effect on IOP. Modification of the drug has resulted in 2 PGF2α analogues that are now in clinical use, latanoprost and unoprostone.

Latanoprost is a PGF2α analogue currently

---

**From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine (Willis, Vitucci, Schmall), and the Center for Biostatistics (Hoshaw-Woodard), The Ohio State University, Columbus, OH 43210. Dr. Willis’ present address is Roweay Memorial Animal Hospital, 171 Union St, Springfield, MA 01105. Dr. Diehl’s present address is Dept of Surgical Sciences, University of Wisconsin, Madison, WI, 53706. Dr. Kobayashi’s present address is 2-35-18 Seijo, Setagaya, Tokyo, Japan 157-0066. Results of this investigation were reported at the 31st Annual Meeting of the American College of Veterinary Ophthalmologists, Montreal, QC, Canada, Oct 11, 2000.**
approved for use in humans with glaucoma. Latanoprost reduces IOP by increasing the uveoscleral outflow of aqueous humor from the eye.\textsuperscript{22,23} Studies of human ciliary muscle cells in tissue culture indicate that PG directly modulate extracellular matrix metabolism, which may be related to increased uveoscleral drainage.\textsuperscript{24} The percentage of aqueous outflow that occurs through the uveoscleral route varies among species as follows: humans (4 to 14%), nonhuman primates (30 to 65%), dogs (15%), cats (3%), and rabbits (13%).\textsuperscript{25-29} Normal drainage of aqueous humor from the anterior chamber of the eye in horses occurs through both conventional and unconventional routes.\textsuperscript{31} Evaluation of the anatomy of the aqueous outflow channels in horses supports the theory that uveoscleral outflow represents a considerable, and perhaps major, pathway for removal of aqueous humor from the anterior chamber.\textsuperscript{32,33} The supraciliary space in ponies is prominent, compared with that in eyes of dogs and primates.\textsuperscript{34} Clinically, medications capable of enhancing this naturally prominent outflow pathway may reduce treatment of glaucoma in this species. The purpose of the study presented here was to evaluate the effect of topical administration of 0.005% latanoprost solution on the IOP of eyes of clinically normal horses and document the occurrence of clinically important ocular adverse effects of the medication in this species.

**Materials and Methods**

Horses—Twenty adult horses (10 geldings and 10 mares) were selected on the basis of absence of clinically important ophthalmic findings and a tolerance of manipulation of the head and periocular area. Horses were acclimated to an indoor stall environment for 48 hours prior to initiation of the study and were handled multiple times during this period to familiarize them with the restraint and periocular manipulation required for subsequently performed procedures.

**Measurement of IOP**—Topical administration of anesthetic (proparacaine hydrochloride 0.5%) was applied to the corneal surface, followed by IOP measurement of both eyes, using an applanation tonometer.\textsuperscript{35} Baseline measurements were taken at defined intervals for 2 days to establish normal variation in IOP during a 12-hour period (7, 9, and 11 AM; 3, 5, and 7 PM). The same observer (AMW) performed all measurements. The tonometer was calibrated daily throughout the course of the experiment. All horses tolerated gentle manual restraint for IOP measurement; therefore, medical sedation and chemical paralysis of the auriculopalpebral nerve were not required. Three readings were obtained from each eye in sequence; each recorded reading was a mean value of 3 to 6 applation readings, with a variance ≤ 5%.

**Drug administration**—On days 3 to 7, 0.1 ml (5 μg) of 0.005% latanoprost solution was instilled into the inferior conjunctival cul-de-sac of 1 randomly assigned eye of each horse. The IOP measurements were then performed bilaterally, as described. On days 8 and 9, no drug was administered, but IOP measurements were continued to establish a return to baseline values.

**Determination of adverse effects**—Adverse effects were recorded prior to each IOP measurement. Vertical pupil size was measured in millimeters, using a clear plastic ruler. Background illumination in the university teaching hospital barn subjectively remained constant during the study period.

Biomicroscopy of the anterior segment was performed, using a hand-held slit-lamp.\textsuperscript{7} The presence or absence of conjunctival hyperemia, epiphora, blepharedema, blepharospasm, and aqueous flare was recorded in both eyes prior to each IOP reading.

**Data analysis**—Recorded IOP of horse eyes at each period was a mean value of the 3 tonometry readings for that eye. Mixed linear regression models were used to compare the IOP of each eye (treated and untreated) for each horse (male and female) at each period (baseline, treatment, follow-up) according to the equation:

\[
Y_{ij} = (\beta_0 + b_i) + \beta_1 \times \text{EYE} + \beta_2 \times \text{SEX} + \beta_3 \times \text{TIME} + \epsilon_{ij}
\]

where \(\beta_0\) is the intercept of this regression line for the jth period (j = 0 [baseline], 1 [treatment], 2 [follow-up]); \(\beta_1\) is the mean difference in IOP between treated and untreated eyes for the jth period; \(\beta_2\) is the mean difference in IOP between the 2 sexes for the jth period; \(\beta_3\) indicates the slope of the regression line for the jth period, where the slope is an estimate of the mean change in IOP for each 1 hour change in time; and \(b_i\) and \(\epsilon_{ij}\) are normally distributed random variables, where \(i\) designates the horse (i = 1...20).

The model assumes a constant correlation among the time points. Regression lines were generated separately for each group at each period (baseline, treatment, follow-up). One overall regression line combining all of the periods was computed to assess the differences between baseline, treatment, and follow-up, controlling for sex and eye (treated and untreated). The mean IOP and SEM were calculated for the subgroups defined by the combination of sex and eye. Plots were created for each sex to illustrate the difference between treated and untreated eyes. Adverse effects (conjunctival hyperemia, miosis, epiphora, blepharospasm, blepharedema, and aqueous flare) were investigated, using frequencies and histograms. Miosis was graded as mild (pupil size < 30% smaller than before treatment), moderate (pupil size between 30 and 60% smaller than before treatment), or marked (pupil size > 60% smaller than before treatment). All other adverse effects were scored as present or absent at each interval. A \(P\) value of < 0.05 was used to indicate significance.

**Results**

**Change in IOP**—Mixed linear regression models indicated an overall difference in IOP between the 2 sexes (\(P = 0.001\)). Mean IOP over time for treated and untreated eyes of geldings was 3.6 mm Hg higher than that of mares. At baseline, there were no differences between treated and untreated eyes (\(P = 0.7\)), nor was there a time effect (\(P = 0.294\)), when controlling for sex.

Mean IOP was significantly lower during the treatment period (\(P < 0.001\)) and follow-up periods (\(P < 0.001\)) than during the baseline period, even after controlling for the difference between sexes. Across sexes, the difference in IOP between baseline and the treatment period was 2.2 mm Hg lower for treated eyes than untreated eyes. The difference in IOP between baseline and the follow-up period was 2.3 mm Hg lower for treated eyes than untreated eyes. Overall, the IOP in treated eyes was 2.3 mm Hg lower than untreated eyes. A significant (\(P < 0.001\)) interaction was found between sex and eye during the treatment period; mean difference in IOP between treated and untreated eyes was 1.08 mm Hg higher for geldings than for mares. Considering all eyes, the difference in IOP between baseline and the treatment period was 2.4 mm Hg lower for mares than for geldings. The difference in
IOP between baseline and the follow-up period was 1.3 mm Hg lower for mares than geldings. Mares had an overall mean IOP reduction of 17%, whereas in geldings the mean IOP reduction was 5%.

During the follow-up period, there was a significant (P < 0.001) time effect such that mean IOP increased 0.07 mm Hg for every hour increase in time (or 1.68 mm Hg/d), when controlling for both sex and eye. Controlling for the effect of time and sex, treated eyes still had a mean of 2.4 mm Hg lower IOP than untreated eyes did during the follow-up period (Table 1).

The mean IOP over time for geldings and mares was plotted to compare treated and untreated eyes within each sex (Fig 1). Separate plots were created for mares and geldings as the result of the overall lower IOP in mares. The plots illustrate the difference between treated and untreated eyes, the decreasing IOP during the treatment period, and the increasing IOP during the follow-up period after drug withdrawal.

### Table 1

<table>
<thead>
<tr>
<th>Time period</th>
<th>Untreated eye</th>
<th>Treated eye</th>
<th>Untreated eye</th>
<th>Treated eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>19.57 (0.72)</td>
<td>19.41 (0.55)</td>
<td>17.38 (0.37)</td>
<td>17.37 (0.39)</td>
</tr>
<tr>
<td>Treatment</td>
<td>21.28 (0.62)</td>
<td>18.38 (0.62)</td>
<td>16.17 (0.38)</td>
<td>14.36 (0.46)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>20.68 (0.94)</td>
<td>18.05 (0.70)</td>
<td>17.04 (0.56)</td>
<td>14.88 (0.55)</td>
</tr>
</tbody>
</table>

**Adverse effects**—Adverse effects in treated eyes associated with topical administration of latanoprost were investigated, using frequencies and histograms. The histograms were combined for all days in the treatment period because of the similar pattern of adverse effects that developed each day. Conjunctival hyperemia was observed in all treated eyes during the treatment and follow-up periods. Aqueous flare, evaluated with biomicroscopy, was not observed in any eye at any
time point. Miosis, epiphora, blepharedema, and blepharospasm were documented in treated eyes variably during the treatment and follow-up periods. Adverse effects were not observed in untreated eyes at any time during the study.

Vertical pupil size decreased from 7 to 11 AM and then increased from 3 to 7 PM. The peak of the drug's miotic effect occurred sometime between 11 AM and 3 PM (4 to 8 hours after treatment). At 11 AM 77% of horses had moderate or marked miosis, by 7 PM (12 hours after treatment) 39% of horses had moderate or marked miosis, and by 7 AM the next day 10% of horses had moderate or marked miosis (Fig 2). There was no significant difference in the pattern of miosis between mares and geldings. Moderate or marked miosis persisted in 5% of horses during the 2-day follow-up period.

The frequency of epiphora increased from 7 to 11 AM (hour 4) and then decreased by 3 PM (hour 8). At 11 AM 57% of horses had epiphora, and at 3 PM (hour 8) only 14% had epiphora (Fig 3). Epiphora was more common in geldings. Only 1 horse had epiphora during follow-up, and that was at 7 AM the first day.

The peak of blepharedema occurred at 3 PM (hour 8), with 12% of horses having signs. There was no significant difference in the occurrence of edema between mares and geldings.

The frequency of blepharospasm increased from 7 to 11 AM (hour 4) and then decreased by 3 PM (hour 8). At 11 AM 42% of horses had blepharospasm, and at 3 PM only 17% had blepharospasm. Geldings had significantly more blepharospasm than mares. Overall, 25% of geldings had blepharospasm versus only 11% of mares. Blepharospasm was not observed in any horse during the follow-up period.

Discussion

Several topically administered medications have been evaluated for their ability to reduce IOP in the eyes of horses. In 1 study, topical administration of 2% pilocarpine twice daily to eyes of clinically normal horses had no significant effect on IOP; however, it did cause ocular irritation. Results of another study suggest that pilocarpine may actually impede uveoscleral outflow of aqueous humor in horses, and a transient increase in IOP has been observed in glaucomatous eyes of horses treated with pilocarpine. Although contraindicated in the management of glaucoma in dogs, topical administration of 1% atropine sulfate has been suggested as treatment for glaucoma in horses because of its enhancement of uveoscleral outflow. Recently, topical administration of atropine was shown to reduce IOP in treated eyes by a mean 11.2% in a group of clinically normal adult horses. However, no significant effect on IOP was observed with short-term administration of atropine to eyes of clinically normal horses in another study, and individual horses have been reported to have increased IOP after atropine use in other reports. Topical administration of a β-blocker, 0.5% timolol maleate, reduced IOP by 17% in eyes of clinically normal horses, compared with pretreatment values, when 1 dose was administered bilaterally. In this same study, application of 0.5% timolol maleate every 12 hours resulted in a 27% reduction in IOP from baseline on the fifth treatment day. Topical administration of the inibiting α-adrenergic system inhibitor dorzolamide alone and in combination with timolol induced only a modest reduction of IOP in eyes of clinically normal horses when administered every 12 hours in another study. Evaluating the effect of other drugs on IOP in horses may provide additional options for treatment of glaucoma in this species. The morphologic features of the aqueous humor drainage pathways in horses, in particular the large capacity for uveoscleral outflow, suggest a drug that increases outflow through this route should theoretically have a therapeutic advantage in this species. The advantages of latanoprost in humans, compared with other glaucoma medications, include a different mode of action, good IOP-reducing effect, once-daily dosing, and absence of systemic adverse effects.

In our study, topical administration of latanoprost reduced IOP in treated eyes of mares and geldings. Mares had an overall mean IOP reduction of 17%, whereas in geldings the mean IOP reduction was just over 5%. The reason for the greater reduction of IOP in mares, compared with geldings, is not known. In a study examining the effect of latanoprost in humans, diurnal IOP in women was reduced 0.7 mm Hg (13%) less than in males (P = 0.001), when controlling for differences in baseline IOP and sex in the ANOVA. The number, sensitivity, and binding capacity of FP-receptors (receptors specific for PGF2α) in the eyes of women and mares may be responsible for the differential effect observed. One possible concern, considering there may be a true sex effect with latanoprost administration, is the effect exogenous PGF2α may have on cycling, pregnant, or periparturient mares, in that PGF2α is the primary luteolytic agent in mares. In humans, latanoprost reaches systemic concentrations that are below the amount expected to stimulate FP-receptors outside the eye, and it is rapidly eliminated with a half-life in plasma of 17 minutes, which explains why clinical trials have not revealed any systemic adverse effects with latanoprost. However, there are no adequate and well-controlled studies of latanoprost use in pregnant women. Reproduction studies have been performed in rabbits, which revealed an inci-
Latanoprost is a highly lipophilic pro-drug, which allows excellent corneal penetration. The pro-drug becomes trapped within the cornea where it is completely hydrolyzed, resulting in a sustained release of the active form of the drug into the anterior chamber. The IOP-lowering effect of latanoprost persists for 20 to 24 hours after a single dose in humans, which allows a single daily dosage regimen. During the 2-day follow-up period in our study when no drug was administered, the treated eyes still had a mean IOP of 2.4 mm Hg lower than untreated eyes, suggesting a short-term persistent drug effect after withdrawal. Despite this, a mean daily increase in IOP of 0.7 mm Hg was observed in treated eyes after drug withdrawal, indicating that at least once-daily latanoprost treatment would also be warranted in horses with glaucoma.

In addition to the desirable response of reduced IOP, latanoprost caused adverse effects in treated eyes of most horses. Miosis was a prominent effect but varied in intensity among horses. Seventy-seven percent of horses had moderate to marked miosis by 4 hours after latanoprost administration. This effect partially subsided by 12 hours after treatment, with only 33% of horses having moderate or marked miosis at that time. Moderate to marked miosis persisted in 10% of horses 24 hours after treatment during the treatment period and 5% of horses during the follow-up period.

In our study, manifestations of ocular irritation were also observed in most of the latanoprost-treated eyes. Conjunctival hyperemia was seen in all latanoprost-treated eyes during the treatment period, whereas aqueous flare was not detected by use of slit-lamp examination in any eye at any time point. Epiphora and blepharospasm were observed in the latanoprost-treated eyes of approximately half of the horses in our study, and blepharedema was observed in a lower percentage (12%) of horses. The peak epiphora, blepharospasm, and blepharedema developed sometime between 4 and 8 hours after latanoprost administration. These adverse effects were attributed to the effect of PG on intraocular and periocular tissues. Miosis was a prominent response in latanoprost-treated eyes of cats and dogs, but other than a mild conjunctival hyperemia, signs of ocular irritation were absent. This difference may be caused by species variation in PG binding by ocular tissues. The dose of latanoprost administered in our study was approximately 3 times the dose typically administered to the eyes of dogs, cats, or humans (the 0.1 ml delivered to our horses was approximately 3 drops from the manufacturer’s bottle). It is possible that a lower dose of latanoprost could reduce IOP without inducing the adverse effects observed in most of our horses.

Two PG molecules have important physiologic and pathophysiologic roles in the tissues of the eye. For example, PGF$_{2\alpha}$ takes part in mediating IOP, whereas PGE$_{2}$ is the primary mediator of inflammation. Miosis may be associated with direct stimulation of the iris sphincter by endogenous or exogenous PG. There are tremendous differences in the responsiveness of the iris sphincter of different mammalian species to the miotic effects of PG. The iris sphincter of cats and dogs contracts in response to PGF$_{2\alpha}$ but not in response to other PG at concentrations that can be expected to develop under physiologic conditions. Although results of in vivo studies on eyes of cattle are not available, results of in vitro studies indicate that the isolated iris sphincter from cattle has a contractile response in the presence of several PG, but that the threshold concentration for this response is about 100-fold higher than the concentration of PGF$_{2\alpha}$ required to cause a similar effect on iris sphincters of cats or dogs. Results of in vivo and in vitro studies also agree that the iris sphincter of several other species, including rabbits and diurnal primates (rhesus and cynomolgus monkeys, baboons, and humans), does not have similar miotic responses to any of the PG that had been studied so far. Miosis is not a commonly reported adverse effect of latanoprost use in humans.

Mild adverse effects associated with latanoprost usage in humans include changes in iris pigmentation, hypertrichosis, hypopigmentation of eyelashes, and mild conjunctival hyperemia. The most common adverse effect observed with latanoprost administration is an increased pigmentation of the iris, mainly in eyes with irides that are already partly brown. This effect is seen with several naturally occurring PG and is caused by stimulation of melanin production in the melanocytes of the iridal stroma. No structural changes of the melanocytes have been observed in studies performed in vivo and in vitro. Although similar adverse effects are possible in horses, they would not be expected to develop in the short period our study entailed. More important reported adverse effects of latanoprost use in humans include cystoid macular edema and anterior uveitis; however, these effects are more likely to develop in individuals with a history of uveitis, prior cystoid macular edema, or previous intraocular surgery. Latanoprost can increase disruption of the blood-aqueous barrier early after cataract surgery in humans. Horses with previous or concurrent uveitis are at increased risk for the development of glaucoma. In our study, breakdown of the blood-aqueous barrier, clinically manifest as aqueous flare, was not observed in treated horse eyes. These horses, however, had no clinical evidence of prior uveitis (synechia, keratic precipitates, chorioretinal scarring) and therefore would be unlikely to incur a spontaneous blood-aqueous barrier breakdown in response to latanoprost. Because most horses with secondary glaucoma appear to be at risk for equine recurrent uveitis, latanoprost treatment has the potential to exacerbate this inflammatory condition. Administration of nonsteroidal anti-inflammatory drug eye drops such as diclofenac sodium seems to prevent the adverse effects of latanoprost treatment in humans.
while maintaining its effect to lower IOP. Because nonsteroidal anti-inflammatory drugs (flubiprofen, flunixin meglumine, phenylbutazone, aspirin) are commonly administered topically and systemically to horses with equine recurrent uveitis, these drugs may be capable of preventing the negative PG-related effects latanoprost may induce.

In addition to its IOP-reducing effect, latanoprost increases optic nerve-head perfusion. Glaucoma in horses can result in extensive and diffuse optic nerve damage, with the normal optic nerve axon density in glaucomatous eyes being reduced by 65%. The axoplasmic flow of the optic nerve in glaucoma is subjected to shearing forces at the scleral lamina cribrosa as a result of the abrupt reduction in hydrostatic pressure between the intraocular and intraorbital spaces. Increased optic nerve head perfusion may be important for retention of visual function in glaucomatous eyes of horses. This effect remains speculative in horses, however, as evaluating optic nerve blood flow was beyond the scope of our investigation.

References

40. Hedman K, Alm A. A pooled-data analysis of three randomized, double-masked, six-month clinical studies comparing the

Correction: Experimental infection of cats with Trichomonas foetus

In the article “Experimental infection of cats with Trichomonas foetus” (AJVR, Nov 2001, pp 1690–1697), the figure should appear in color as follows:

Figure 5—Photomicrograph of immunohistochemical analysis for T foetus antigen in colonic mucosal biopsy specimens obtained from cats, using monoclonal antibody TF1.15 (dilution of 1:500, incubated 60 minutes at 37 C). Panels A and C are cytocentrifuged preparations of T foetus (NCSU Tfs-1) cultured from a cat with large-bowel-type diarrhea. Trichomonads were fixed in formalin and centrifuged (300 X g for 5 minutes) onto poly-L-lysine-coated slides prior to immunohistochemical staining. Slides were treated identically, except for omission of primary antibody for panel C (negative-control specimen). Surface immunolabeling of trichomonads is evident in panel A, but notice that background staining is not evident in panel C. Panels B and D are similar tissue sections obtained from a cat experimentally inoculated with T foetus. Sections were treated identically, except for omission of primary antibody for panel D (negative-control specimen). In panel B, immunolabeled trichomonads (red-stained organisms) are located on surface enterocytes and within superficial mucus and detritus of the cecal mucosa. Antigen also is evident within the superficial epithelia and lamina propria. Notice that background staining is not evident in panel D. Immunostained by streptavidin-biotin-peroxidase with 3-amino-9-ethylcarbazole as the chromogen and methyl green counterstain. Bar = 100 µm.