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OCULAR FINDINGS AND SELECT OPHTHALMIC DIAGNOSTIC TESTS IN CAPTIVE AMERICAN WHITE PELICANS (*PELECANUS ERYTHORHYNCHOS*)

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Abstract

The aim of this study was to establish normal ophthalmic parameters for select diagnostic tests in American white pelicans (*Pelecanus erythrorhynchos*). Twenty-one zoo-housed American white pelicans were manually restrained for noninvasive ocular diagnostic testing and complete ophthalmic examination. Tear production quantification using the phenol red thread test (PRTT), fluorescein staining, and intraocular pressure (IOP) evaluation were performed. In addition, conjunctival aerobic bacterial culture and culture-independent 16S rRNA amplicon sequencing were performed on select eyes. Normal variations and ocular abnormalities detected during complete ophthalmic examination were documented and photographed. Direct pupillary light reflex, menace response, and palpebral reflex were present in all birds. The value (mean $\pm$ SD) for PRTT and IOP was 14.9 ± 7.84 mm/15 sec and 9.0 ± 1.41 mm Hg oculus uterque, respectively. Conjunctival culture in nine birds revealed no growth for six birds and *Staphylococcus aureus* growth in three birds. A high relative abundance of *Mycoplasma* sp. was detected in all samples based on 16S rRNA sequencing. The normal pelican eye was found to have relative conjunctival hyperemia, absent filoplumes, iris color ranging from light blue to brown, and a subcircular vertically elongated pupil. Ophthalmic abnormalities were noted in 10 of 21 birds. Common

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findings included corneal fibrosis, cataracts, and asteroid hyalosis. The most common ophthalmic abnormality in this species was cataracts.

Keywords

Eye; intraocular pressure; microbiota; ocular; pupil

INTRODUCTION

Avian species represent a considerable portion of animal collections in zoologic parks worldwide. However, limited data exist to characterize normal ophthalmic examination findings and typical ophthalmic diagnostic test results for most avian species. Descriptive and quantitative ophthalmic studies have been published for some bird orders in an effort to disseminate baseline values in birds commonly maintained in zoologic collections. The intent of these studies is to provide veterinary clinicians with normal ophthalmic diagnostic test values in various avian species. This information assists in the diagnosis and treatment of ocular conditions in avians, ultimately resulting in improved veterinary care and subsequently enhanced welfare for avian species.

The American white pelican (*Pelecanus erythrorhynchos*) is popular in zoologic collections, and many detailed studies have been performed to better understand their physical peculiarities. Published studies evaluating the ocular system of pelicans include a summary of ophthalmic diagnostic and examination findings in brown pelicans (*Pelecanus occidentalis*),²⁷ the quantification of the refractive state in air and water of brown pelicans,³² and a clinical description of a penetrating keratoplasty in a juvenile brown pelican whereby a full-thickness donor allograft was placed into a full-thickness defect of a recipient pelican that survived 6 wk postoperatively.²¹ To the authors' knowledge, no published data exist characterizing ophthalmic examination findings or normal ranges for ophthalmic diagnostic tests in the American white pelican.

The purpose of the present study was to describe ocular examination findings in a collection of zoo-housed American white pelicans and to define objective ocular diagnostic test parameters. An additional aim was to characterize the conjunctival ocular microbiota of American white pelicans by using standard aerobic bacterial culture as well as culture-independent methods.

MATERIALS AND METHODS

Twenty-one adult American white pelicans (9 females, 12 males) housed at the Saint Louis Zoo and deemed nonreleasable due to permanent injuries were manually restrained for physical and ocular examination. Sex of the birds was determined using polymerase chain reaction (PCR). Blood was collected from the medial metatarsal vein in five birds for complete blood count and plasma biochemistry. An Institutional Animal Care and Use Committee approval from the Saint Louis Zoo was obtained before project commencement. All birds were deemed healthy based on a physical examination performed under manual

restraint by a board-certified zoo veterinarian (MEK) before being evaluated by a board-certified veterinary ophthalmologist (JWP).

Basic ophthalmic diagnostics and examination

Objective ophthalmic diagnostic testing was performed in 41 eyes of 21 birds; one eye was excluded due to marked phthisis bulbi. For the phenol red thread test (PRTT), the sterile phenol red thread (Zone-Quick, Showa Yakuhin Kako Co Ltd, Tokyo, 104-0031, Japan) was placed routinely (Fig. 1A) and read after 15 sec. Fluorescein staining (Ful-Glo, Akron Inc, Lake Forest, Illinois 92630, USA) and intraocular pressure (IOP) evaluation by rebound tonometry (TonoVet, iCare, Finland Oy, Ayrity 22 Tuiki, FI-01510, Vantaa, Finland) were also performed. For re-bound tonometry, the “P” manufacturer calibration setting was used because this species did not have internal calibration data available.²⁴ Three sets of IOP measurements were taken for each eye, and the results were averaged and reported as a single reading. A complete ophthalmic examination, including slit-lamp biomicroscopy and indirect ophthalmoscopy, was performed without pupillary dilation after objective diagnostic testing in all birds ($n = 21$). Extraocular photographs were obtained for all eyes ($n = 42$).

Bacterial culture and microbiota characterization

Standard aerobic bacterial culture was performed in nine eyes of nine randomly selected birds. A culturette (Remel BactiSwab, Starplex Scientific Inc, Etobicoke, Ontario M9W 6Y3, Canada) was used to swab the upper and lower conjunctival fornix of the right eye (OD). Microbiota characterization was performed in 20 eyes of 20 birds. The pelican with the eye with phthisis bulbi was excluded from this testing method. A single sterile cotton tip applicator was used to swab the upper and lower conjunctival fornix of the left eye (OS). After collection, the swabs were immediately placed into a sterile plastic vial and submitted to the MU Metagenomics Center (Columbia, Missouri 65211, USA). The DNA extraction and analysis were performed based on previous studies.^{10,11} Bacterial 16S rDNA amplicons were constructed via amplification of the V4 hypervariable region of the 16S rDNA gene with universal primers (U515F/806R), flanked by Illumina standard adapter sequences.^{6,34} Oligonucleotide sequences are available at proBase.²⁰ A single forward primer and reverse primers with a unique 12-base index were used in all reactions. PCR reactions (50 µl) contained 100 ng of genomic DNA, forward and reverse primers (0.2 µM each), dNTPs (200 µM each), and Phusion High-Fidelity DNA Polymerase (1 U). PCR amplification was performed as follows: 98°C(3:00) + [98°C(0:15) + 50°C(0:30) + 72°C(0:30)] × 25 cycles + 72°C(7:00). Amplified product (5 µl) from each reaction was combined and thoroughly mixed; pooled amplicons were purified by addition of Axygen AxyPrepMagPCR Clean-up beads (Corning Life Sciences, Tewksbury, Massachusetts 01876, USA) to an equal volume of 50 µl of amplicons and incubated at room temperature for 15 min. Products were washed multiple times with 80% ethanol, and the dried pellet was resuspended in EB buffer (32.5 µl, Qiagen, 19300 Germantown Rd, Germantown, MD 20874, USA), incubated at room temperature for 2 min, and then placed on the magnetic stand for 5 min. The final amplicon pool was evaluated using an advanced analytical fragment analyzer automated electrophoresis system, quantified with a Qubit fluorometer using the quant-iT HS dsDNA reagent kit (ThermoFisher Scientific, Waltham, MA 02451 USA), and diluted according to Illumina's standard protocol for sequencing on the MiSeq. Assembly, binning, and

annotation of DNA sequences was performed at the MU Informatics Research Core Facility. In brief, contiguous DNA sequences were assembled using FLASH soft-ware²² and culled if found to be short after trimming for a base quality less than 31. Qiime v1.8¹⁷ software was used to perform de novo and reference-based chimera detection and removal, and the remaining contiguous sequences were assigned to operational taxonomic units (OTUs) via de novo OTU clustering and a criterion of 97% nucleotide identity. Taxonomy was assigned to selected OTUs by using BLAST¹ against the Greengenes database⁸ of 16S rRNA sequences and taxonomy.

Statistical analysis

All statistical tests were performed using SigmaPlot (Systat Software Inc, San Jose, California 95110, USA). Data were subjected to the Shapiro–Wilk test to test for normal distribution and determine whether standard parametric tests could be used. When evaluating the relationship between IOP and sex of the bird, data were not normally distributed, and a nonparametric test was used instead. The Fisher exact test of independence was used to determine whether there was a relationship between eye color and sex of the birds. For PRTT and IOP, a paired *t*-test was used to test for significant differences between OD and OS values with respect to each measure. The PRTT values from the OD and OS were averaged for each bird, and a *t*-test was used to determine whether there was variation in this value based on sex of the birds. For IOP, values from the OD and OS were averaged for each bird, and a Mann–Whitney rank sum test was used to look for differences between male and female birds.

RESULTS

Basic ophthalmic diagnostics and examination

Vision (positive menace response) and direct pupillary light reflex were present in both eyes (OU) of all birds except for a single bird with advanced unilateral phthisis bulbi. Indirect pupillary light reflex was absent in all birds. A palpebral reflex, characterized by excursion of the nictitating membrane over the entire globe and narrowing of the palpebral fissure, was observed in all birds; a complete blink was not noted. For PRTT and IOP, there was no statistically significant difference between eyes, so OD and OS values were combined. The mean PRTT of animals with a normal ophthalmic examination was 14.9 mm OU, with an SD of 7.84 mm. All birds were fluorescein negative OU. The mean IOP of animals with a normal ophthalmic examination was 9.0 mm Hg OU, with an SD of 1.41 mm for all birds. The PRTT and IOP values did not show any statistically significant variation between eyes or sex of the birds.

Ophthalmic examination revealed yellow periocular skin lacking feathers with multiple folds and invaginations (Fig. 1). All eyelid margins were devoid of filoplumes. The nictitating membrane was semitransparent. The color of the iris varied from blue (Fig. 1B) to brown (Fig. 1C). The majority of birds had blue iris color OU ($n = 16$), but a small number had brown irises OU ($n = 4$). One bird had complete heterochromia iridis, with one blue and one brown iris. Iris color was independent of sex in the birds. The conjunctiva was relatively hyperemic compared to other domestic mammals and bird species (Fig. 1D). The pelican

pupil was ellipsoid with subtle vertical elongation (Fig. 1). The fundus was gray-brown with a chocolate-brown pleated pecten oculi extending into the vitreous humor. A well-demarcated fovea was not consistently visible on indirect ophthalmoscopy, but it was detected in the inferior nasal quadrant OU in three birds.

Approximately half of the birds ($n = 11$) had a normal ophthalmic examination without any ophthalmic abnormalities detected. A single bird exhibited moderate subcutaneous emphysema of the periocular tissue of the left eye (Fig. 2A). One bird also had a skin tag-periocular mass in the adnexa measuring less than 1 mm. Marked unilateral phthisis bulbi was observed in a single bird (Fig. 2B), with trauma suspected as an etiologic diagnosis. Subtle microphthalmia was noted in another bird that also had incipient cataracts and corneal fibrosis OU. Early phthisis bulbi was considered unlikely in this bird but could not be definitively ruled out due to the unknown history and because the small globe size could have been either congenital (microphthalmia) or acquired (phthisis bulbi). Microphthalmia was suspected due to the symmetrically small globes and the absence of other ophthalmic abnormalities. Four birds (six eyes) had corneal lesions, with three of these birds (five eyes) having corneal fibrosis and one bird having a corneal foreign body located superficially in the superior paraxial cornea OD. The foreign body was less than 1 mm in size and was easily removed using a cotton-tipped applicator. One of the birds had corneal fibrosis in the same eye as periocular emphysema; historic trauma was a suspected cause for both these abnormalities. A small atypical iris coloboma was noted in the pupillary zone of the iris at 9 o'clock OD in one bird (Fig. 2C). Cataracts were the most common abnormality detected and were present in 19% of the birds (four birds, seven eyes). Anterior cortical incipient cataracts were present in two birds (four eyes), and nuclear fibrillar incipient cataracts were present in one bird (two eyes). A late-stage resorbing hypermature cataract was present OS in one bird; this eye also exhibited increased conjunctival hyperemia due to suspected low-grade uveitis, although active uveitis (aqueous flare) was not detected in this eye (Fig. 2D). Asteroid hyalosis was observed in one eye of one bird. A circular, depigmented retinal scar or retinal pigment epithelial coloboma measuring one quarter of the pecten width was located in the inferior nasal fundus of the bird with marked phthisis bulbi in the contralateral eye.

Bacterial culture

Aerobic culture results reported from the right eye of nine birds revealed no growth ($n = 6$, 67%) and light *Staphylococcus aureus* growth ($n = 3$, 33%). The sensitivity results were unremarkable with all cultured *S. aureus* sensitive to the following antibiotics: amikacin, ampicillin, ciprofloxacin, gentamicin, oxacillin, trimethoprim-sulfa, neomycin, marbofloxacin, amoxicillin-clavulanic acid, doxycycline, azithromycin, enrofloxacin, cefazolin, cefpodoxime, ceftazidime, cefovecin, cephalixin, and orbifloxacin.

Microbiota characterization

Based on an average coverage of 14,752 sequences per sample, the conjunctival microbiota of all birds was dominated by *Mycoplasma sp.*, which accounted for $81.38 \pm 11.82\%$ of the extracted DNA. Although an average of 91.3 ± 31.08 distinct OTUs (groups of sequences sharing a minimum of 97% nucleotide identity) were detected in any individual sample, the

majority were present at very low relative abundance, with only nine other OTUs approaching or exceeding 0.5% mean relative abundance (Fig. 3). After *Mycoplasma* sp., the two most abundant genera were *Deinococcus* sp. and *Corynebacterium* sp., detected at 2.58 ± 3.62 and $2.11 \pm 2.15\%$ relative abundance, respectively. Of note, DNA specific for unclassified *Staphylococcus* sp. was detected in 18 (90%) of 20 eyes at a relative abundance of $0.10 \pm 0.10\%$.

DISCUSSION

The family Pelecanidae contains eight species of birds with similar morphology. To date, limited information regarding the pelican eye is available, with studies primarily focusing on the brown pelican. To the authors' knowledge, this is the first study documenting ophthalmic exam findings and diagnostic test parameters in the American white pelican. A case report describing brown pelican documented IOPs of 11 mm Hg (OD) and 7 mm Hg (OS) by using applanation tonometry and an inability to evaluate aqueous tear production because of the small conjunctival cul-de-sacs and lack of tolerance by the awake bird during attempted application of the tear test strip.²¹ For birds in the present study, a similar range for IOP was documented, but in contrast, measurement of tear production was readily achievable using the PRTT. Examination findings from a group of 63 brown pelicans have also been documented previously.²⁷ In this group of brown pelicans, 46% had a normal ocular examination, similar to the findings in our study (52% were normal). In addition, the most common ophthalmic finding in the brown pelicans was cataract, which was also the case in the American white pelicans presented here. Moreover, similar to the birds in our study, several of the brown pelicans also had vitreal degeneration, corneal disease, and globe damage due to suspected trauma. Lack of filoplumes was noted in brown pelicans²⁷ and the American white pelicans in this study and seems to be a common feature in birds with long bills, possibly allowing unobstructed views of the rostral bill during feeding.

The pneumatic system of pelicans is extensive.³⁰ Prominent periocular subcutaneous emphysema was documented in the present study, suggesting that subcutaneous air space may be capable of extending to the space immediately adjacent to the eyes.

Four birds (six eyes) had corneal lesions, with the majority of the birds ($n = 3$; five eyes) having corneal fibrosis or scarring that was most likely due to traumatic injury and similar to a previous report in brown pelicans.²⁷ One of the birds had corneal fibrosis in the same eye as periocular emphysema; historic trauma was suspected.

An interesting finding was the diversity of iris color, ranging from blue to brown in the American white pelicans in this study. Eye color did not statistically correlate with sex of the birds. The birds in this study were adults of unknown age. Brown pelican juveniles have a brown iris that transitions to tan or blue during the breeding season and returns to brown after incubation.²⁷ In other avian species such as the bald eagle (*Haliaeetus leucocephalus*), iris color changes with advancing age.¹⁸ A correlation between age and iris color was suspected, but it could not be confirmed in the present study due to lack of age data.

The ellipsoid shape with vertical elongation of the pelican pupil is subtle, albeit a significant finding, because most avian species have round pupils.⁹ Another bird reported to have a vertically elongated pupil is the black skimmer (*Rynchops niger*),³⁷ a nocturnal and crepuscular feeder. American white pelicans also exhibit both diurnal and nocturnal feeding during the breeding sea-son.²³ Correlation between vertical pupil elongation and nocturnal ambush feeding in reptiles and mammals is well supported.^{3,5} A vertically elongated pupil facilitates appropriate retinal illumination in a variety of light conditions by protecting the retina in bright-light environments.^{3,5} For the American white pelican, this would facilitate both diurnal and nocturnal for-aging. In addition, a vertically elongated pupil results in a larger depth of field that assists many ambush predators in locating prey.³

One bird in this study was diagnosed with a small unilateral atypical iris coloboma. Iris colobomas were reported as inherited, sex-linked defects in a flock of rosecomb bantam chickens.⁷ The iris coloboma noted in a single pelican in this study does not support an inherited, flock-based problem, but further studies would be warranted to confirm this possibility.

Cataracts were detected in 19% of the birds ($n = 4$; seven eyes) and were the most common ophthalmic finding in this study. These lens abnormalities were not considered to be clinically or functionally significant except for in one eye of one bird with suspected subclinical uveitis associated with a hypermature cataract. The presence of cataracts in captive raptors and other birds is well documented in other studies and is often a common ophthalmic finding.^{14,18,19,24} A late-stage resorbing hypermature cataract was present in the OS in one bird. Unfortunately, refraction was not performed in this patient to confirm hyperopia due to limited access to this equipment in the field. The underlying etiology for both the incipient and hypermature cataracts is unknown. Traumatic etiology was suspected for the hyper-mature cataracts because the birds had been free ranging and were maintained in the collection due to musculoskeletal injuries. Senile etiology was considered a possibility for the incipient cataracts, although the ages of the birds could not be determined in this study. Ocular evaluation of free-ranging scops owls (*Otus scops*) and little owls (*Athene noctua*) admitted for blunt force trauma revealed corneal erosions-ulcers and cataracts to be common.³¹

Asteroid hyalosis was documented in one bird in this study and has previously been documented in a bald eagle and brown pelicans.^{18,27} The age-related development of vitreous degeneration was suspected in both previous studies, because only the adult brown pelicans had vitreous degeneration and the single eagle in the case report was geriatric.

In the American white pelicans in this study, the fundus appearance and foveal location (when observed) in the inferior nasal quadrant were consistent with previous historic reports of other Pelicaniformes.^{27,35}

Tear production can be measured in avian species by using a Schirmer tear test I or a PRTT. We selected PRTT instead of a Schirmer tear test because many zoologic medicine clinicians prefer PRTT for use in avians because the small-diameter thread allows for easier placement into small palpebral fissures, and the measurement time of 15 sec is preferable in manually

restrained birds. The PRTT values (mm/15 sec) in American white pelicans (14.9 ± 7.84) are similar to those documented in screech owls (*Megascops asio*) (15 ± 4.3)¹⁴ and Hispaniolan parrots (*Amazona ventralis*) (12.5 ± 5.0)³³ and slightly lower than values previously reported in large psittacines (range = 19.1–28.2)³⁶ and American flamingos (*Phoenicopterus ruber ruber*) (24.2 ± 4.4).²⁴

Rebound tonometry was selected for IOP measurements in this study because it is rapid, does not require topical anesthesia, can be performed on eyes with corneas as small as 1.4 mm in diameter, and is routinely used in avian and exotic animal species.^{2,14,15,24–26,29} There is a need for species-specific reference intervals for IOP in birds, because broad ranges for different species have been reported historically.²⁹ Variations in avian IOP parameters are attributed to differences in corneal thickness that positively correlate with IOP in raptors.⁴ The corneal thickness of pelicans was not evaluated in the present study. In addition to evaluating the corneal thickness, a study comparing the IOP in different pelican species may be interesting given their morphologic similarities, but differing feeding strategies. The rebound IOP measurements obtained in American white pelicans in this study (9.0 ± 1.41 mm Hg) were similar to those reported in other avian species by using the uncalibrated (p) setting.^{15,19,24,26} In addition, they were also similar to those obtained via applanation tonometry in normal American brown pelicans (10.86 ± 1.61 mm Hg).²⁷

Aerobic culture of the conjunctival sac has been performed in several bird species with variable results. The present study highlights potential limitations of routine aerobic culture when attempting to characterize the bacterial flora of the conjunctival sac, because six of nine cultures had no bacterial growth and three of nine cultures documented a single bacterium, *S. aureus*. These results are consistent with reports in other avian species.^{24,36} Because state-of-the-art technology is now available to evaluate microbiota via PCR and sequencing analysis,^{10,11} we sought to more completely characterize the microbiota of the pelican conjunctiva by using this highly sensitive, culture-independent method. The identification of a rich microbiota in the conjunctival fornix was not unexpected, although we were surprised to detect such a consistently high relative abundance of a single genus, *Mycoplasma* sp. Although certain species of *Mycoplasma* have been studied for their pathogenic potential in avian hosts, including conjunctivitis in house finches (*Haemorrhous mexicanus*) and other songbirds,^{12,16,28} the high mean relative abundance detected in this cohort of otherwise healthy pelicans suggests that certain mycoplasmal species are benign residents. Also of note, DNA specific for *Suttonella ornithocola* was detected in 17 of 20 samples. This species was first isolated from the lungs of deceased birds from the tit families and was speculated to be the cause of death;¹³ the present data suggest that it may be a normal part of the ophthalmic microbiota of some avian hosts. Regarding the positive culture results for *S. aureus* in three of nine samples tested, culture-independent methods detected DNA specific for *Staphylococcus* sp. in 18 of 20 samples at a mean relative abundance of 0.10%. Thus, it is possible that culture was not sufficiently sensitive to detect colonization with *S. aureus* in other birds, or that other uncultivable *Staphylococcus* spp. were present.

The intent of the present study was to describe ophthalmic exam findings, establish reference ranges for tear production and IOP, and characterize typical conjunctival microbiota in American white pelicans. Ultimately, the hope is that the data provided here

will aid in identification and management of ophthalmic diseases in this captive avian species.

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Figure 1.

Extraocular photographs of normal American white pelican eyes. An example of phenol red thread placement is shown in (A). Blue iris coloration is demonstrated in (B) and brown iris coloration in (C). Typical appearance of bulbar and palpebral conjunctiva with relative hyperemia is demonstrated in (D).

Figure 2. Extraocular photographs of American white pelican eyes demonstrating ocular pathology including periocular emphysema OS (A), phthisis bulbi (B), iris coloboma highlighted by white arrow (C), and late-stage resorbing hypermature cataract (D).

Figure 3.

Bar chart demonstrating the relative abundance of the 10 most prevalent operation taxonomic units (OTUs) detected in conjunctival swabs collected from captive American white pelicans ($n = 20$). Legend at right.