Bilateral ocular anomalies in a South African fur seal (*Arctocephalus pusillus pusillus*)

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Abstract

A female South African fur seal (*Arctocephalus pusillus pusillus*) began having obvious clinical ophthalmologic problems by 8 weeks of age. The initial clinical sign was diffuse corneal edema, which progressed to bullae formation and ulcers; the underlying cause of corneal edema and bullous keratopathy was not identified antemortem. An ophthalmological evaluation was performed when the fur seal was approximately 6 months of age, but due to the diffuse corneal edema, intraocular structures could not be easily evaluated. An underlying infectious etiology was suspected; therefore, appropriate diagnostics were pursued, but did not identify a cause. Initial improvement was noted, but the fur seal then became blind and eventually became very painful. Due to decreased quality of life and aggressive behavior, the fur seal was euthanized. Histopathological diagnoses were persistent tunica vasculosa lentis and persistent hyperplastic primary vitreous with bilateral hypermature resorbed cataracts and retinal detachments with rosette formation.

Key Words: cataract, embryology, ocular anomaly, PHPV/PHTVL, pinniped, zoo and exotics

INTRODUCTION

This is the second report of bilateral ocular anomalies in a young pinniped. The first animal is alive and was evaluated via ophthalmologic and ultrasonographic examination, but without histological evaluation. In the present report, we were able to evaluate the eyes histologically and compare them with the antemortem clinical findings.

CASE REPORT

A female South African fur seal (*Arctocephalus pusillus pusillus*) was born as the 4th offspring from a 12-year-old female kept at the Rostock Zoo for the past 6 years. The Rostock Zoo keeps a collection of three females and one male South African fur seals. All female animals were born under human care and were acquired from the Frankfurt Zoo, Germany, whereas, the 16-year-old male was born in the wild and was acquired from an animal dealer from South Africa. There are one to two pups born each year in the collection. No ocular abnormalities had been observed at this facility prior to this report. The keepers had noticed that the fur seal pup had ‘blue eyes’ beginning at approximately 8 weeks of age, although she appeared to thrive normally, and was at an appropriate weight for her age, and did not behave as if her vision was functionally impaired. At approximately 10 weeks of age, the fur seal was briefly evaluated without magnification by the co-author (JCR) and diagnosed with diffuse bilateral corneal edema, and the surface of the cornea had a stippled effect with multifocal tiny raised foci consistent with bullae. As the evaluation was without magnification, corneal bullae suspicion could not be confirmed. In addition, the fur seal’s eyes appeared painful based on behavior to avoid manipulations of eyes and administration of soluble topical ophthalmic medications, which included 0.3% ofloxacin ophthalmic solution (Floxa Film, Bausch & Lomb GmbH, Berlin, Germany) and 0.25% hyaluronic acid lubricating solution (I-Drop, I-Med Pharma, Dollard-des-Ormeaux, QC, Canada) were both given BID. The treatment was nonspecific and aimed at a potential infectious keratopathy; the underlying initiating cause was suspected to be...
due to a potential viral infection with secondary bacterial infection. The co-author (JCR), who is the zoo veterinarian for this facility and also primarily practices ophthalmology, was not alerted to any other problems with the fur seal until over 3 months later.

At approximately 6 months of age, the fur seal was sedated using midazolam (Dormicum 5 mg/mL, Roche Pharma AG, Grenzach-Wyhlen, Germany) at a dose of 0.25 mg/kg given intramuscularly (IM), and a complete ophthalmological examination was performed. Dazzle reflex was present OU. Schirmer tear tests were presumed normal at 25 mm/min OU (both eyes); superficial corneal ulcers were diagnosed using fluorescein staining and slit-lamp evaluation. Diffuse moderate corneal edema was present with a few bullae throughout the superficial corneal epithelium. Pinnipeds typically have miosis pupils in ambient light; therefore, pupillary light responses (PLR) were not elicited; the moderate corneal edema might have precluded clear view of minor movements by the pupil margins. Although fine detail of the anterior chamber was not possible, no abnormal structure formation, such as extensive persistent pupillary membranes, hyphema or hypopyon, or a mass effect occupying the anterior chamber, was seen OU. Fundic examination was impossible. Intraocular pressures were measured at 31 mmHg OD and 32 mmHg OS with a Tono-Pen VET™ (Reichert Technologies, Depew, NY, USA); this value is within the normal range for pinnipeds.² Treatment with 0.3% ofloxacin ophthalmic solution (Floxal®, Bausch & Lomb GmbH) and 0.25% hyaluronic acid lubricating solution (I-Drop®, I-Med Pharma) were continued, and 5% sodium chloride ophthalmic solution (OmniSorb, Omni-Vision GmbH, Puchheim, Germany) was added. All topical medications were given TID. Two weeks later, the fur seal’s eyes appeared to be improving; the corneal epithelium was smooth and intact, but severe edema remained although bullae had resolved.

Two weeks following the previous recheck, the fur seal was reported to have been bumping into objects for the previous 7–10 days. On brief evaluation with only physical restraint, using biomicroscopy, the anterior chambers could not be examined with diagnostic detail due to severe corneal edema. Again, no corneal bullae were evident, and the epithelium was smooth and intact.

Two days later, the seal was sedated again with midazolam (Dormicum 5 mg/mL, Roche Pharma AG) at a dose of 0.25 mg/kg IM with poor response. After 30 min, medetomidine (Domitor®, 1 mg/mL, Orion Corporation, Espoo, Finland) was administered a dose of 10 µg/kg IM combined with butorphanol (Torbugesic® 10 mg/mL, Pfizer GmbH, Berlin, Germany) at a dose of 0.08 mg/kg IM. She was easier to handle but still attempted to bite. Sedation with midazolam may have been impaired by factors including not injecting directly into the muscle or variability with increased agitation and/or pain level that was higher than the first time the fur seal was sedated (J. Bailey, personal communication veterinary anesthesiologist with extensive marine mammal experience). Ophthalmologic findings included a positive dazzle reflex OU, fluorescein staining was negative OU, and intraocular pressures were measured at 23 mmHg OD and 32 mmHg OS (within normal limits). The corneas had moderate diffuse edema. There were no bullae present, the anterior chambers were slightly visible, and the pupils were mid-dilated OU (Fig. 1). Mid-dilated pupils in a pinniped are considered mydriatic under dark lighting conditions and abnormal in ambient light; direct and consensual PLRs could not be elicited in either eye. Conjunctival samples were collected using cytobrush for virological testing, and blood/serum was submitted for serological testing. All samples were submitted to the University of Giessen, Germany, where pan-Herpesvirus PCR was performed and was negative. Serological testing for canine distemper virus and canine adenovirus was also negative. As an infectious etiology was highest on the differential list, samples were collected and submitted for aerobic bacterial and fungal organisms (Idexx VetMed Labor, Ludwigsburg, Germany). An ultrasound evaluation was not considered at this time due to the higher likelihood of infectious causes.

Topical ophthalmic medications continued with 0.3% ofloxacin ophthalmic solution (Floxa®, Bausch & Lomb GmbH) and 0.25% hyaluronic acid (I-Drop®, I-Med Pharma), and 0.1% diclofenac sodium ophthalmic solution (Voltaren® Ophtha, Novartis Pharma GmbH, Nuremberg, Germany) was added; all were given TID OU, pending results of diagnostic testing. Cultures collected for aerobic bacteria and fungi were negative, but medications were continued.

Figure 1. The right eye of the fur seal with diffuse corneal edema. The iris can be distinguished and is mydriatic for a pinniped in ambient light.

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After 14 days of using this regimen, the zookeepers reported that the fur seal had become disoriented and very aggressive. The pup refused to feed from her mother and had bitten the keepers numerous times. Due to the diminished response to therapy, very poor quality of life, and poor prognosis, the fur seal was euthanized and full necropsy performed. Tears, conjunctival tissue, aqueous humor, and conjunctival cells were collected and frozen for potential testing based on histopathological results. Eyes were collected in 10% buffered neutral formalin and submitted for pathological evaluation.

**Necropsy results**

Small stones and a metal screw were found in her stomach, but no other systemic abnormalities were found, other than the eyes.

Culture and sensitivity from conjunctival samples for aerobic bacteria and fungi, collected at necropsy, were negative.

The right globe measured 35 mm axial diameter measured from the anterior corneal surface to posterior surface of the sclera, 34 mm horizontal equatorial diameter and 34 mm vertical equatorial diameter. The left globe measured 32 mm axial diameter measured from the anterior corneal surface to posterior surface of the sclera, 40 mm horizontal equatorial diameter and 40 mm vertical equatorial diameter. Eyelids and adnexal structures, including third eyelids, were normal OU. Corneal epithelium was thickened and acanthotic; there was diffuse edema throughout the stroma. In the deeper stromal layers, there was vascularization anterior to Descemet's membrane, and the endothelium was normal. There were no inflammatory cells evident.

The anterior iridal surfaces and iridocorneal angles, OU, were covered by a pre-iridal fibrovascular membrane (Fig. 2) causing entropion uvea and blocking the iridocorneal angle, respectively (Fig. 3). The ciliary body and its musculature were normal. Both lenses were smaller than normal, with lens capsule wrinkling, and hypermature cataract formation with presumed resorption. There was a thin, pigmented fibrotic membrane anterior to the lens, that is, the tunica vasculosa lentis (Fig. 4). The posterior capsule was ruptured. An extracapsular plaque of vascularization was present adjacent to the equatorial lens capsule (Fig. 5) and posterior to the cataractous lens capsule, that is, the tunica vasculosa lentis and the primary vitreous.

The choroid and the tapetum were normal. Both eyes had complete retinal detachments, and the retinas showed dysplastic rosettes (Fig. 6). The surface of the retina was also covered by a fibrovascular membrane. Optic nerves OU were normal.

**DISCUSSION**

The developing eye requires oxygen and nutrients supplied by a complex vascular system. The hyaloid vascular system consists of the tunica vasculosa lentis anteriorly, primary vitreous posteriorly, and the hyaloid artery. These are
anatomically described as separate entities; however, the components are structurally inter-related by rich anastomoses and functionally inter-related in their development and regression. Under normal conditions, the anterior-most vascular network, the pupillary membrane regresses due to capillary endothelial cell apoptosis, likely induced by macrophages. Regression of the hyaloid vasculature system may be triggered by retinal angiogenesis and growth of the globe. The molecular cause is not well understood, but mediation by the WNT pathway activated by macrophages may be involved. Support for this hypothesis involves Ninjurin1 expression on macrophages, which induces apoptosis of the hyaloid vasculature through the WNT pathway. A newer report reveals that autophagy, a nonapoptotic cell death pathway, is also involved in regression of the hyaloid vascular system along with caspase-dependent apoptosis during hyaloid regression.

Persistence of the hyaloid vascular system occurs due to failure of its appropriate regression and is termed persistent hyperplastic tunica vasculosa lentis and persistent hyperplastic primary vitreous (PHTVL/PHPV). These uncommon congenital ocular anomalies have been reported in dogs, mice, humans, speculated to be the case in another young pinniped. The exact underlying cause(s) of PHTVL/PHPV have not been identified. A line of transgenic mice with mutated macrophages resulted in persistence of the pupillary membrane and the hyaloid vasculature. Homozygous mice, in another line of transgenic mice with an unidentified mutation, also developed PHTVL/PHPV, in addition to having a high incidence of histiocytic sarcomas (40%, C.M.H. Colitz, personal observation). The authors hypothesized that the histiocytes, including the hyalocytes, were abnormal; another possibility was that the transgene inserted into a gene important for ocular development. The gene was never identified; therefore, we can only speculate on these mechanisms.

Persistent hyperplastic primary vitreous results in posterior capsular cataract, retrolental fibrovascular membrane, leukocoria, and, in some cases, secondary retinal detachment. As in other reported cases of PHPV/PHTVL, both of this patient’s eyes had fibrovascular membranes and rudimentary vascular remnants posterior to the lens. In addition, this case had pre-iridal fibrovascular membrane formation on the anterior iridal surfaces and iridocorneal angles, causing entropion uvea and blocking the iridocorneal angle. Pre-iridal fibrovascular membrane formation occurs secondary to uveitis, trauma, intraocular surgery, glaucoma, and retinal detachment due to production of cytokines, especially vascular endothelial growth factor (VEGF). With retinal detachment, the hypoxic retina secretes VEGF and this promotes proliferation of new vascular growth in the iris, ciliary body, and optic nerve head. Secondary glaucoma may ensue due to the peripheral anterior synchia and the fibrovascular membrane covering the iridocorneal angle or due to iris bomb. With so many changes within the globes in this case, the cause of the fibrovascular membranes may have been directly related to the PHPV/PHTVL and/or to VEGF-related production. IOPs were not measured as elevated at either examination suggesting that there might have been areas of aqueous humor outflow in areas of the iridocorneal angle that were not in the sections evaluated or that there might be use of the uveoscleral pathway.

In the present case, we can only speculate about the course of events, based on the literature describing eyes with PHPV/PHTVL. Initially, the lens may have been clear but would have become cataractous due to rupture of the posterior capsule with resulting invasion of the fibrovascular tissue. In addition, the retinas may not have been detached at this time. This may explain why the fur seal was functionally sighted initially. Menace responses were not elicited at any time; however, we do not know at what age the response is learned in this species. The fur seal tried to avoid...
obstacles and trainers initially, which would indicate functional sight. A rapidly ensuing cataract may have caused lens swelling and, with time, the cataractous lens was resorbed, leaving a wrinkled capsule and a shrunken cataract, as were found histopathologically. Measurement of IOPs was only possible with sedation. Midazolam, used initially, has not been shown to significantly change IOP\textsuperscript{17}, and IOPs were not abnormal at that time; however, medetomidine, an alpha-2 agonist, which lowers IOP,\textsuperscript{18} was used at the subsequent examination, possibly lowering IOPs, although they were still in the normal range. We discuss this because the globes differed in measurements, and there were fibrovascular membranes covering the iridocorneal angles. The OS was larger in width and height than OD, although both were similar in length. A normal age-matched South African fur seal pup globe was not available for comparison. Therefore, it is not known if one globe was abnormally large, or the other had acquired or congenital microphthalmia.\textsuperscript{19} Microphthalmia can occur in eyes with PHPV/PHTVL,\textsuperscript{4} and the optic nerves in this fur seal were normal histologically, making glaucoma unlikely.

Diffuse corneal edema with bullous keratopathy is not typically seen with PHPV/PHTVL. Histologically, there was vascularization evident anterior to Descemet’s membrane, although the endothelium was intact in the sections evaluated. The corneal vascularization, without obvious cause, may have been responsible for the diffuse edema, or there may have been areas of lost or disrupted corneal endothelial cells not evident on studied sections.\textsuperscript{20} Prior to the histological information, juvenile otariid keratopathy was considered to be the cause of diffuse edema and bullous keratopathy was secondary to the edema.\textsuperscript{21,22}

Diffuse corneal edema with or without bullous keratopathy is common in pinnipeds with otariid keratopathy.\textsuperscript{23} In the author’s experience (CMHC), there are two scenarios for corneal bullae in pinnipeds. The first is in pups or juvenile otariids, typically younger than 3–4 years of age, and the presentation is bilateral and very slow to improve (up to a year or longer) despite aggressive treatment. The definitive underlying cause of this specific manifestation is unknown, although we still cannot rule out a viral cause; therefore, diagnostics for infectious etiologies were pursued. Treatments for diffuse edema with bullous keratopathy in juvenile otariids have included oral antiviral medication, that is, famcyclovir; various topical and oral antimicrobials to prevent secondary infections as well as MMP-induced stromal loss, that is, doxycycline or minocycline; hypertonic (5%) saline drops, gels, or ointments; topical and oral nonsteroidal anti-inflammatory and narcotic medications for pain, when present, and lastly, topical cyclopentolate or taclolimus drops. Recently, 50 parts per thousand (5%) salinated water in small pools were provided to young affected sea lion pups overnight for days to weeks, and the affected eyes responded rapidly and were completely normal within 2–3 months. The manifestation in the juvenile otariids is different from the adult otariid keratopathy where bullous lesions are extremely uncommon. Affected adults initially present similarly with diffuse edema and multiple bullae; however, most improve rapidly with topical and oral antimicrobials including doxycycline or minocycline, other antibiotics and antifungal medications, nonsteroidal and narcotic pain medications, and hypertonic saline solution. These cases often present following excessive rainy periods or changes in chlorine or ozone (C.M.H. Colitz, personal observation). The results of a current epidemiological analysis including over 300 pinnipeds in 25 facilities worldwide will help us to identify the water-related and other environmental risk factors important to maintain corneal health in pinnipeds.

The relatively mydriatic pupil seen in the latter examination and images was retrospectively attributed to retinal detachment. Histologically, there were bilateral retinal detachments with dysplastic rosette formation. In some cases of PHPV/PHTVL, the retina may be detached either by congenital nonattachment or by traction from fibrils of the primary vitreous inserted into the sensory retina.\textsuperscript{24,25} This may result in dysplasia, folds, or accumulations of reactive retinal pigment epithelial cells.\textsuperscript{4,24} The surface of the retina, in this case, was covered by a fibrovascular membrane; therefore, this may have been the cause of secondary retinal detachment and rosette formation. Retinal dysplasia may also be associated with variable persistence of the hyaloid vasculature.\textsuperscript{26} It is also possible that the rosettes were due to retinal dysplasia not associated with the PHPV/PHTVL secondary retinal detachment. Unfortunately, it is not possible to know whether there were one or two separate ocular disorders.

Incidence of PHTVL/PHPV is typically unilateral in humans, although bilateral cases are associated with genetic conditions such as trisomy 13/15 and others.\textsuperscript{9} In the Staffordshire Bull Terrier and the Doberman Pinscher, as well as transgenic mice, the condition is inherited and bilateral.\textsuperscript{12,27–29} Similar to this case, the other reported pinniped, a phocid, had bilateral congenital anomalies without diffuse corneal edema.\textsuperscript{1} The fur seal in this report is the first case of an offspring born of its parents’ matings having an identified congenital anomaly. As pinnipeds are predisposed to corneal disease,\textsuperscript{30} we were not suspicious of a congenital anomaly. This pup was seen to have ‘blue eyes’ 2 months after birth, as per the zookeepers; therefore, the time of onset was unknown. This was eventually clinically diagnosed as diffuse edema with bullous keratopathy. Based on this, the age of the animal, and the fact that no other pinniped was having corneal problems at the time, differential causes included viral or traumatic with secondary bacterial infection. Water quality was not highly suspected as the underlying cause of the corneal disease. Chlorine is kept lower than what is typically acceptable, as is the pH. It is possible that if the water were salinated, the edema and bullae may have been somewhat improved. A previous unpublished epidemiological study found that there was a strong association for
otariids in freshwater to have corneal edema (L. Dunn, personal communication, 1996 IAAAM proceedings). The co-author (CMHC) concurs with this finding; however, if the corneas are healthy, otariids do not have an increased incidence of edema. While there is no shade provided for the fur seals at this facility, the ultraviolet index is very low and they are far north of the equator. The author (CMHC) has observed that there is increased corneal disease in animals closer to the equator and in locations that have high ultraviolet indices.

CONCLUSIONS
In summary, this case demonstrates a young pinniped with bilateral PHTVL/PHPV. However, the bilateral nature of this congenital condition in our case, and congenital anomalies seen in another young phocid, may suggest either a genetic disease or spontaneous somatic mutation in these species.

REFERENCES


