

Clinical case report of unilateral idiopathic keratitis in a bottlenose dolphin (*Tursiops truncatus*)

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Abstract

Keratitis also called corneal ulcer is inflammation of the cornea, an ocular disorder that can lead to severe visual impairment and requires prompt diagnosis and treatment to avoid complex sequelae that can range from corneal opacity to corneal scarring, perforation, endophthalmitis and loss of the eye. Ulcer types are classified into superficial ulcer, deep ulcer, neurotrophic ulcer, perforating ulcer and corneal erosion. The objective of this work is to describe the clinical case of an adult female bottlenose dolphin (*Tursiops truncatus*) that was under human care belonging to the company Delphinus, which presented symptoms such as blepharitis and closed eye, characteristic symptoms of keratitis, confirming the diagnosis in this specimen was a challenge due to the retraction of the eye, however, The treatments used for this condition ranged from antibiotics and anti-inflammatories to alternative procedures such as the use of plasma rich in growth factors and ophthalmic ozone. With the latter, recovery of the integrity of the corneal epithelium and a minimal formation of scar tissue was observed; the definitive diagnosis was confirmed once the specimen opened its eye.

Keywords

Corneal inflammation • Keratitis • Ophthalmic ozone • Corneal ulcer

Introduction

Bottlenose dolphins (*Tursiops truncatus*) can develop ophthalmic lesions usually involving the cornea and often traumatic in origin. Under human care they tend to develop a variety of diseases that are influenced by their environment, such as increased exposure to sunlight (ultraviolet radiation); air pollution; changes in water quality parameters including unbalanced salinity, pH, chlorine and/or ozone, as well as disinfection by-products; and Changes in coliform counts and probably other bacteria or yeasts or fungi that we do not yet control.

Materials and Methods

Factors that negatively influence dolphin vision can lead to acute pain resulting in severe blepharospasm, in which there is no possibility of evaluation or administration of medications, if the eye does not open within 48 hours they must be treated orally. Severe pain resulting in blepharospasm is due to an infected corneal ulcer, blunt or sharp trauma, or a significant change in water quality. Keratitis is the name given to inflammation of the cornea regardless of etiology or severity [1].

Anatomy and physiology

The cornea histologically comprises 5 layers. The first is the epithelium, which is a non-keratinized stratified squamous type, consisting of 4 to 7 layers of cells, which flatten as they become more superficial. Below this is the basement membrane (BM) and Bowman's membrane, which is an acellular layer made up of randomly located collagen fibers; here are the nerve endings that give the epithelium. It does not regenerate if damaged (Bowling, 2016a). The stroma continues which is the thickest layer, it represents 90% of the total corneal thickness (Fernandez et al., 2008) and is formed by collagen fibers arranged in parallel, which gives the characteristic of transparency to the cornea. Below the stroma is Descemet's membrane, formed by several types of collagen and organized in a hexagonal shape, which gives it great resistance. Finally, the endothelium is the deepest layer, consisting of a monolayer of hexagonal cells unable to regenerate, its pumping function maintains an adequate state of hydration of the overlying layers, it is also important for maintaining the transparency of the cornea (Figure 1).

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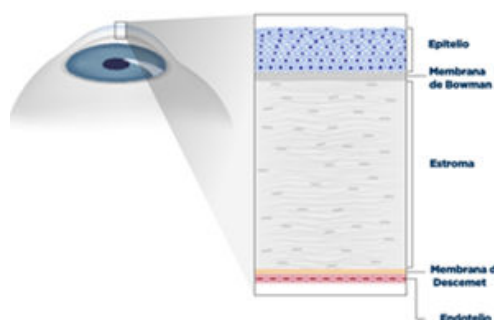


Figure 1. Structure of the cornea, with its different layers (from outside to inside) epithelium, Bowman's membrane, stroma, Descemet's membrane and endothelium. Upper part tear film, lower part aqueous humor, by Sheperd Eye Center.

Pathophysiology and healing

The process by which the cornea presents an inflammatory reaction is defined by its avascularity; this causes the inflammatory phenomena to derive from the sclerocorneal limbus and finally ends in corneal neovascularization. Another main characteristic of the cornea is its transparency, which is lost during keratitis since its proper structure and hydroelectrolytic balance is altered [2].

The cells of the corneal epithelium migrate and multiply to cover the defect, at a rate of 1mm per day. Epithelial turnover occurs by multiplication of stem cells located in the corneal limbus and their centripetal migration. In some animals, complete renewal of the corneal epithelium takes approximately 7 to 10 days. Uncomplicated epithelial ulcers heal without associated fibrosis, leaving no scar in most cases. Bowman's membrane has little regenerative capacity, which explains the recurrence of some corneal erosions when this membrane is affected. An indicator of this incomplete regeneration is the inability of the epithelium to be adequately moistened by the tear film, therefore, dry spots appear and the tear film breaks prematurely. From this layer onwards, any condition will result in corneal opacification and irregularities causing irregular astigmatism and, if it affects the pupillary area, it will also reduce vision.

In the stroma, keratocytes multiply and differentiate into fibrocytes, capable of synthesizing collagen and other components of the extracellular matrix. In turn, in stromal aggressions, chemotactic factors are released that attract initially polymorphonucleated inflammatory cells and vessels, responsible for remodeling the lesion. Most stromal healing processes resolve with fibrosis secondary to the disorganization of collagen fibers [3].

Descemet's membrane requires the presence of endothelial cells for its production and thickening. The corneal endothelium lacks mitotic capacity, so its dead cells must be replaced by migration and hypertrophy of cells adjacent to the lesion. There are many internal and external factors that influence these healing mechanisms, which can chronify or complicate the lesions.

Etiology

- Infectious: Bacteria, viruses, parasites, opportunistic fungi.
- Non-infectious: physical, chemical, immune-mediated.

Results

Classification, signs and symptoms

They are classified according to the time of evolution in simple, complicated and progressive depending on the scarring that can occur between 8 and 10 days, or occur with delayed healing related to infections or other processes, up to a complication by growth and corresponding deepening.

Classification	Characteristics, signs and symptoms
Grade I	Lesion with rounded contours, edematous borders, absence or scarce stromal infiltrate, mild pain in proportion to the defect.
Grade II	Ocular pain, hyperemia, photophobia.
Grade III	Severe episcleritis, corneal opacity and severe neovascularization, moderate photophobia, itching and ocular discharge.

Table1. Classification of corneal ulcers according to clinical presentation.

Clinical diagnosis	Affected corneal layers	Result
Superficial ulcer or superficial stromal	Involvement of the entire epithelium and less than ¼ of the thickness.	Simple / progresiva
Corneal erosion	Epithelium, basement membrane (BM) and Bowman's membrane	Refractory/recurrent
Shallow ulcer	Epithelium, MB, Bowman's membrane, stroma involvement between ¼ and ½	Simple/ progressive
Deep or stromal ulcer	Epithelium, MB, Bowman's membrane, involvement between ¾ and 1 of the stroma.	Complicated progressive /
Descemetic ulcer or desmatocoele	MB, Bowman's membrane and stroma (risk of perforation)	Complicated progressive /
Perforating ulcer	Rupture of endothelium, outflow of aqueous humor, fibrin formation and iris prolapse.	Progressive

Table2. Types of corneal ulcerations.

Diagnosis

Identifying the cause or contributing factors requires a complete eye examination. The eyes should always be evaluated for abnormalities in eyelid structure and function and preocular tear film disorders.

Fluorescein is an orange stain that changes to green under alkaline conditions, is highly lipophobic and hydrophilic. It is used to observe the integrity of the corneal surface. It has the particularity of

staining only the corneal stroma, a quality transmitted by its exclusively hydrophilic staining capacity; so that if the cornea has natural lipid coverage it does not adhere and does not stain. In conclusion, if it stains it is because the integrity of the corneal epithelium has been lost. On the contrary, it does not stain when Descemet's membrane is compromised because it is hydrophobic. But it is not difficult to notice because the crater is very typical and the almost vertical edges of the crater, which are part of the stroma, are stained [4].

Several studies suggest the use of rose bengal in ophthalmology, because it stains the nucleus of dead cells, being then together with fluorescein, the most used dyes to detect damage to the corneal epithelium, such as dry eye and ulcerative keratitis. If possible, cytology, culture and sensitivity samples can be collected, and treatment should be guided by the results of these tests.

Medical treatment

Cetaceans have a very thick tear film rich in mucus. For this reason, topical ointments are not recommended. Suspensions or ophthalmic drops are recommended. Treatment depends on the cause and should be initiated as soon as possible to prevent further corneal injury.

In addition, the use of topical 3% acetylcysteine prior to medication (or mixed with medication) appears to help the medication(s) enter the tear film easier and possibly create a deposit of that medication in the tear film.

Antimicrobial therapy

Oral doxycycline is typically used with topical antibiotics, and the latter is used in this combination to treat opportunistic flora and ulcer-infecting bacteria, if known. Enrofloxacin has good activity against Gram-positive and Gram-negative bacteria, including some pathogenic anaerobes.

The combination of oral doxycycline (4-5 mg / kg BID until the eye is open and often until the cornea has healed) and an oral quinolone such as enrofloxacin (2.5 mg / kg BID for 5-7 days) is the authors' suggestion for treating suspected corneal infections when severe blepharospasm is present.

Gentamicin is indicated for the treatment of ocular infections caused by Gram-positive and Gram-negative bacteria, as well as for conjunctivitis, blepharitis, iritis, uveitis, keratoconjunctivitis, keratitis, trauma and ocular postoperative therapy; there are combinations with ketorolac where it exerts an anti-inflammatory, local analgesic and antipruritic effect, its mechanism of action is due to its ability to inhibit prostaglandin biosynthesis [5].

Tobramycin is a broad-spectrum bactericidal aminoglycoside antibiotic that *via* topical route is indicated in the inflammatory process of the anterior segment of the eye, associated with superficial bacterial infection or with risk of infection, its combination with dexamethasone, a corticosteroid inhibitor of inflammatory response, is frequently used for inflammatory ocular conditions where there is a risk of bacterial ocular infection.

Pain management

Tramadol is an opioid used for severe pain, for pain in the ophthalmologic sphere, this pain can cause a high concern in the patient, given the important role that vision has in their quality of life.

Trypsin and chymotrypsin as an enzymatic compound act by unfolding inflammatory mediators and degranulation of plasma proteins and immunocomplexes displaced to the tissue through their direct dissociation by stimulating phagocytosis. All this contributes to pain relief (Quiles, 1965).

Alternative therapy

PRGF (Plasma rich in platelet growth factors) is an autologous concentration of dolphin platelets in a plasma volume that represents an increase in platelets with respect to normal basal concentrations. It comes from the specimen's own blood, so it cannot cause hypersensitivity reactions and is free of transmissible diseases. It is obtained after centrifugation of unclotted whole blood and is composed of plasma, leukocytes, platelets and growth factors, but the most important elements are platelet-derived growth factors, transforming growth factor beta, insulin-like growth factor, fibroblast growth factor, vascular endothelial growth factor and epidermal growth factor that exert the regenerative function. In addition to repairing the wound it regenerates lost tissues. It has been used as an adjuvant in corneal lesions progressing to stromal ulceration [3].

Ozone (O₃)

Ozone (O₃) is a molecule consisting of three oxygen atoms (O₂), has proven, consistent and safe therapeutic effects. Its mechanism of action is by inactivation of a large part of microorganisms, stimulation of oxygen metabolism and activation of the immune system. The gaseous forms of medication are somewhat unusual, and it is for this reason that special application techniques have had to be developed for the safe use of ozone, the side effects are minimal and preventable.

Ozone therapy is generally used as an adjuvant to conventional treatments, either systemically or locally. Minor autohemotherapy (AHM-m) is considered as the practice in which venous blood is extracted and mixed with ozone for subsequent intramuscular injection. The immunological action of this gas on the blood is fundamentally directed on monocytes and T lymphocytes, which once induced, release small amounts of practically all cytokines, so the release will occur in an endogenous and controlled manner. This regulation is given because ozone acts as an immune system enhancer by activating neutrophils and stimulating the synthesis of some cytokines [2].

Surgery

Surgical repair of deep stromal ulcers, descemetocoeles or perforations has not yet been performed in cetaceans, but is an option; however, it may be difficult to address these problems in most facilities in an emergency. Therefore, prevention is the best option.

Description of the site

Delphinus Riviera Maya is located within Xcaret Park, a park located on the Chetumal to Puerto Juárez highway km 282 in Playa del Carmen, Quintana Roo, Mexico.

The use of ozone in certain pathologies of the anterior segment of the eye could be providential due to its anti-inflammatory and bactericidal activity, in addition to promoting tissue repair properties. In particular, there is a need for new products for the treatment of ocular pain and inflammation, such as during external ocular infections or inflammations, due to the related risk of blindness. In addition, ozone allows for "physiological" wound healing, minimizing the risk of keloid scarring and also the risk of corneal turbidity [5].

Case analysis

Female bottlenose dolphin *Tursiops truncatus*, 27 years old, weighing 182 kilograms and 267 centimeters in length. This animal was in a pond of considerable size shared with 4 other specimens of the same species. The conditions and quality of the water were checked by the veterinary team, which constantly quantified colony-forming units and monitored temperature and salinity daily, as well as the health and physical condition of the specimen. Its diet consisted of capelin, herring, squid and gelatin (Figure 2).



Figure2. Bottlenose dolphin (*Tursiops truncatus*) specimen. Delphinus (2019)

Anamnesis

For the treatment of an oral fistula presented by the dog, physical containment (handling) was performed daily for the assessment and cleaning of the lesion. On July 17, 2019, during one of these treatments, the veterinary team reported the right eye closed, ten days later the conjunctiva of the lateral canthus of the right eye was observed hyperemic, and treatment was performed with apparent improvement.

However, on July 30, 2019 the eye is reported totally closed with mild blepharitis, on several occasions stalks are reported near her right upper eyelid and blepharitis, different therapies are performed, the specimen opens her eye on September 12, 2019 but continues with maintenance therapy until October 2, 2019 [1].

Clinical findings

It is noted the improvement of an oral fistula that was being treated and the right eye closed with retraction of this, hyperemic lateral canthal conjunctiva, blepharitis of the upper eyelid and superficial stalks around the eye.

Diagnostic aids

Semiology, biometry and blood biochemistry without relevant alterations for the case. Fluorescein test in the left eye negative and in the right eye inaccurate result, evident ulcer scar.

Treatment

At the first report of the closed right eye, in order to prevent endophthalmitis or to attack bacteria, treatment was started with Tobracetil® eye drops (acetylcysteine 44.17mg and tobramycin 3mg) three TID drops for four days with the aid of a syringe connected to a TomCat probe (Front cover, n.d.), a small size probe, with a hole in the distal end that allows fluid drainage; followed by a subconjunctival injection of gentamicin 0.1ml mixed with physiological saline solution (ssf) 0.9ml, for three alternate days, these were performed with the aid of palpebral separators or Desmarres retractors (Venenhaken, n. d.), it was observed that there were three TID drops during four days with the help of a syringe connected to a TomCat probe (Front cover, n.d.). d.), it was observed that there was retraction of the eye and the conjunctiva of the lateral canthus was hyperemic; for the procedure of subconjunctival injections it was first necessary to apply local anesthetic eye drops, followed by embrocation, injection and finally chloramphenicol 10mg ointment. For pain management she was given tramadol 100mg orally SID [4].

On July 28, 2019, ophthalmic treatment was started with plasma rich in growth factors (pgrf) 0.5ml (extracted from the same specimen days before), mixed with Dolo-Vet® eye drops containing a broad-spectrum antibiotic (gentamicin sulfate 5mg) combined with a non-steroidal analgesic and anti-inflammatory (ketorolac 5mg) 0.5ml with the aid of a syringe and TomCat probe to administer this mixture inside the SID eyeball for ten days.

Simultaneously, Doxycycline and Enrofloxacin were prescribed orally for the oral fistula. During the twelve days of treatment the animal showed apparent improvement and the eye was slightly open, but with blepharospasm (Figure 3), after this time the animal lasted three days with apathy and did not respond to calls from the caregivers and did not allow any review or treatment and its appetite decreased; when it was possible to approach the animal, the right eye was totally closed again. A fluorescein test was performed on the left eye with negative results (Figure 3).



Figure3. Bottlenose dolphin (*Tursiops truncatus*) with half-closed right eye, blepharitis and stubble on the upper eyelid. Sepúlveda, M. (2019).

After the second report of the closed eye, treatment with a mixture of ophthalmic drops and pgrf was resumed again with the help of a probe, since the eye was totally closed, once a day for eight consecutive days; compresses were applied once a day for eight

consecutive days; compresses were applied once a day for eight consecutive days; compresses were applied once a day for eight consecutive days; compresses were applied once a day for eight consecutive days. The affected eye area was treated with cold compresses three times a day and tramadol SID was administered orally for 5 days in a row as pain management. A blood sample was taken (Figure 4).



Figure4. Specimen with evident closed eye, and syringe connected with the small TomCat probe. Serrano, L. (2019)

According to the symptomatology of the specimen regarding the buccal fistula and its eye, oral therapy with trypsin 45,000UNF and chymotrypsin 9,000UNF Ribotrypsin® was started.

Additionally, a minor autohemotherapy was established, 5 ml of blood was extracted from the specimen, mixed with a dose of ozone-oxygen in an ozone concentration of less than 60 µg/ml, then an intramuscular injection was given weekly for a month [3].

When this new treatment was started, the animal improved in terms of mood, but its eye remained closed. On September 2, 2019 a management was performed, where the veterinary team used the palpebral separators to try to observe the eye but the specimen continued with the eye retracted, it was decided to start a less invasive therapy, it was ozone in gas directly to the surface of the eye, at therapeutic concentrations between 20 and 60 µg/ml, The patient opened her eye eight days after starting this therapy, and a fluorescein test was performed with an inaccurate result since the little staining that was observed was presumed to be the final phase of the healing of the ulcer in the cornea.

Three days later a slight blepharitis was noted, so TobraDex® (dexamethasone 1mg, tobramycin 3mg) TID eye drops were applied for five consecutive days, and cold compresses were applied between one and three times a day for ten days in a row.

The specimen responded very well to this last treatment and on September 20, 2019 all the prescribed drugs for both her eye and oral fistula were suspended, leaving as maintenance therapy the use of ophthalmic ozone and cold compresses for 12 more days. Ozone was used for a total of one month.

At the end of the treatment there was a decrease of scar tissue in the cornea of her affected eye (Figure 5).



Figure 5. Maintenance therapy with ozone gas directly to the affected eye. López, K. (2019)

Discussion

Corneal ulcer is an ocular disease that tends to course with severe complications or sequelae and can compromise visual function ("Treatment of severe corneal ulcer with fortified eye drops," 2018), however, in the case described, constant monitoring of the evolution of the specimen with the treatments performed was performed, precisely to avoid unfavorable consequences in her eye.

The accommodation of the eye in the orbit seems to be managed by the contraction of the so-called "retractor bulbi muscle", which produces axial displacements of the eye in the orbit (Supin et al., 2001). It is very complicated to visualize a closed cetacean eye when opening its eyelids (Gulland et al., 2001), in this case the specimen always made ocular retraction, a method that can allow the animal to open the eye to examine it is using a local anesthetic, spraying the liquid with a syringe, in the orbital fissure from a few centimeters away, if it reaches the cornea it will temporarily relieve the pain (Gulland et al., 2001) in this way it can be easier to make a diagnosis.

According to different authors (Baraboglia, 2009; Bron et al., 2003) fluorescein and rose bengal are the most commonly used stains in ophthalmology. In this case fluorescein staining could not provide a definitive diagnosis due to the characteristics of the specimen (retraction), preventing the application of the stain and once healed, fluorescein will not stain any structure, therefore, another diagnostic method should be used.

According to the symptomatology seen in the animal and the experience they had previously had in the dolphinarium, the veterinarians were able to orient themselves by differential diagnoses of keratitis. According to the clinical presentation of corneal ulcers it can be classified as grade II (Gelatt, 2003) and as it took more than two months for recovery or evolution time it can be classified as complicated (Gelatt, 2003). Intense pain that produces blepharospasm is due to an infected corneal ulcer, blunt or acute trauma, or a significant change in water quality (Gulland et al., 2001). However, because this condition occurs in only one individual in the pond, because it manifests unilaterally and because water quality control parameters such as salinity, temperature and colony-forming unit culture are constantly checked, it is ruled out that the water condition was the cause of the keratitis.

Eyes with acute pain resulting in severe blepharospasm, in which there is no possibility of evaluation or administration of medications, should be treated orally (Gulland et al., 2001), in this case it was

decided to use in first instance a compound of acetylcysteine and tobramycin *via* ophthalmic route through a Tomcat probe inside the eyeball to reach the eye, followed by subconjunctival injections of gentamicin, in order to control a possible infection or to avoid the appearance of opportunistic microorganisms.

Antibiotics can be chosen empirically or specifically (Gelatt et al., 2007), the latter was the way the treatment was chosen, since early diagnosis of the condition the specimen was suffering from was impossible. Tetracyclines are the most immunomodulatory antibiotics, promote corneal wound healing and clinically stabilize the corneal stroma during infections where stromal loss is prominent (Gulland et al., 2001). Doxycycline was administered orally together with enrofloxacin at the first report of the ocular lesion for 5 days.

Platelet-rich plasma was shown to be effective for the regeneration of deep and extensive corneal ulcers. In addition, its easy accessibility, cost and reduced dosage are highlighted. Scar tissue does not recover the mechanical properties or physiological function of the tissue or organ that has been damaged, the use of PRGF is to regenerate, rebuild shape and restore function (Acosta et al., 2014). After this treatment with PRGF, ophthalmic ozone therapy and the use of cold compresses were performed, good results were evidenced there, this is attributed to them for being non-invasive therapies, there was a decrease in the inflammation of the upper eyelid and acceleration in the healing process, to result in the opening of the affected eye, these effects obtained determined to continue with the application of the treatment as maintenance therapy, to prevent the specimen from closing the eye again because of inflammation or as a defense mechanism. In this last therapy, a reduction of the corneal ulcer scar was observed.

Conclusion

The diagnosis of a corneal ulcer is usually easy to make, however, this case represented a real challenge for the veterinary team, since the dolphin managed to retract the eye most of the time and it was not possible to perform eye observation, staining or microbial culture,

so it was necessary to use diagnostic guidance tools to establish the appropriate treatments. It is not believed that the quality of the water was the cause of the condition, despite this, it was not ruled out that the keratitis could evolve into endophthalmitis or infection by opportunistic microorganisms, so in the first instance and throughout the treatment broad spectrum antibiotics were used; among other predisposing factors that could affect the integrity of the cornea of the specimen is attributed to trauma, for its duration and response to treatment. We resorted to use alternative therapies such as ophthalmic ozone, with which the results were effective, there was an opening of the eye, minimal scar tissue formation was evidenced and recovery of the integrity of the corneal epithelium, this can be a therapy to include among the treatments for keratopathies or keratitis of any etiology.

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