Received: 1 February 2020 | Revised: 2 September 2020 | Accepted: 27 September 2020

DOI: 10.1111/vop.12837

CASE REPORT

WILEY

Atypical ocular Chelonoid herpesvirus manifestations in a captive Loggerhead turtle (*Caretta caretta*)

Arianne P. Oriá[1] | Danielle N. Silva[1] | Ana Cláudia Raposo[1] | Alessandra Estrela-Lima[1] | Thaís T. Pires[2] | Marco A. Gattamorta[3] | Roberta R. Zamana[3] | Eliana R. Matushima[3] | Ron Ofri[4]

[1]School of Veterinary Medicine and Zootechny, Federal University of Bahia, Salvador, Brazil

[2]Fundação Pró-TAMAR National Coordination, Salvador, Brazil

[3]Laboratory of Wildlife Comparative Pathology, Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

[4]The Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel

Correspondence
Arianne P. Oriá, School of Veterinary Medicine and Zootechny, Federal University of Bahia, Avenida Adhemar de Barros, 500 – Ondina, CEP: 40170-110 Salvador, BA, Brazil.
Email: arianneoria@ufba.br

Abstract

A captive loggerhead turtle (*Caretta caretta*) of unknown sex, 3 years of age, presented with bilateral mucoid secretions, severe chemosis, conjunctival hyperemia, and globe retraction. The animal was evaluated ophthalmologically and systemically, and hematological, microbiological, and conjunctival cytological and biopsy samples were collected for complementary diagnosis. The histopathological examination showed amphophilic intranuclear inclusions associated with severe inflammatory infiltrate. The diagnosis of *Chelonid alphaherpesvirus* 5 (ChAHV 5) was confirmed with end point PCR. Following systemic treatment with L-lysine, acyclovir and vitamin A, the ocular signs resolved. No amphophilic intranuclear inclusions were seen in a follow-up biopsy 5 months later, and there has been no recurrence of clinical ophthalmic signs during a 4-year follow-up. It is suggested that ChAHV 5 be considered as a differential diagnosis in captive marine turtles that present for conjunctival disease other than fibropapillomatosis.

KEYWORDS

Alphaherpesvirus, Chelonia, conjunctivitis, end point polymerase chain reaction, lysine, sea turtle

1 | INTRODUCTION

The loggerhead turtle (*Caretta caretta*) is classified as a critically endangered to vulnerable species, depending on the geographic region.[1] It has a wide geographic distribution, and its habitat includes subtropical and temperate coastal regions.[1] Brazil is the major reproductive niche of the species in the south Atlantic and has a number of national conservation centers to study the animals.[2,3] Due to the importance of vision for this species, recognition and diagnosis of ophthalmic diseases are crucial to its preservation.[4]

Herpesviruses affect different classes of vertebrates and may coexist with their host for long periods of latency.[5] Alphaherpesvirinae subfamily viruses, including chelonid alpha herpesvirus (ChAHV) types 1, 5, and 6, have been described in species of the order Testudines, which includes turtles, tortoises, and terrapins.[6,7] Compared to other reptilian taxa, there is a high incidence of herpesvirus infection in this order, and the virus is isolated from both diseased and healthy animals.[8] Chelonid herpesvirus infection in sea turtles has been implicated in the gray patch and lung-eye-tracheal diseases, which are associated with ChAHV1 and ChAHV6, respectively.[6]

The most common clinical ocular manifestation of ChAHV5 infection in sea turtles is fibropapillomatosis.[5] However, there are also reports of ChAHV5 isolation in

healthy loggerhead turtles.[3,8] This may be because of the complex, multifactorial etiopathogenesis of herpetic disease, which involves environmental factors such as pollutants and water temperature, as well as the degree of virulence of the etiological strain.[2,5,6] Indeed, juvenile loggerhead turtles have a lower incidence of herpetic disease, since as prejuveniles they live in open seas (pelagic), and only as juveniles and adults do they move to coastal areas (neritic) where they have greater exposure to anthropic actions and environmental alterations.[2] However, due to a different habitat and a developing immune system, juvenile loggerheads have a higher incidence of disease, similar to that reported in the green sea turtle.[3,7]

Less common ocular manifestations of herpetic disease have been reported, but to the best of our knowledge these reports are restricted to descriptions of clinical signs, with no data provided on diagnostic workup, treatment, or follow-up.[4,9] Here, we report on an atypical case of ChAHV5 in a captive loggerhead turtle presenting with bilateral chemosis, conjunctival hyperemia, and mucoid secretions. Clinical, hematological, microbial, cytological, histopathological, and end point PCR findings, treatment, and follow-up are described.

2 | CASE HISTORY AND COMPLEMENTARY DIAGNOSTIC TESTS

The study was conducted at the TAMAR Project Center, Mata de São João, Bahia, Brazil (lat. 12.5694 S, long. 37.9887 W). The Center's tanks comply with the published requirements for care and maintenance of captive sea turtles.[10] The animals lived in a tank not covered, located in an outdoor area. The tank's area and volume were 119.7 m^2 and 102 000 L, respectively, and its depth 0.6 to 1.25 m. Tank water is taken from sea and filtered using sand filter, with 100% of the water renewed daily. The salinity and temperature of the water are maintained at 30 ppm and 27-30ºC, respectively. Chlorine is used to disinfect the tanks when they are washed (1-2 times a week) and is also added to the water to reduce organic matter (0.5 ppm daily). The tanks are vacuumed daily to remove stool and other debris. The water from the tanks is filtered and returned to the sea free of chlorine. The animals' diet is based on fish offered inside the tank, with the leftovers removed after feeding. Animals also receive seaweed or vegetables and vitamin supplementation twice a week.

In May 2016, keepers noted signs of ophthalmic disease in a 3-year-old loggerhead turtle (curved carapace length 54.6 cm, weight 19.2 kg) of unknown sex that was kept in an enclosure with five more animals from the same hatching. Clinical signs included bilateral mucoid secretions and chemosis which were more pronounced in the right eye, conjunctival hyperemia, and bilateral globe retraction. Blepharospasm (especially of the left eye) was also noted, though its extent seemed to be limited by the severe chemosis (Figure 1). No other periocular or ocular surface abnormalities were seen, and fluorescein staining was negative in both eyes.

A comprehensive physical examination by the attending veterinarian detected no other abnormalities, and there was no evidence of lethargy or weight loss. All hematological and biochemical parameters were within normal range for the specie and age[11,12] (Table S1). Complete physical and ophthalmic examinations, as well as complete blood count and biochemistry panel, were conducted on other four *Caretta caretta* tank mates (Table S1). Results of these tests were unremarkable, and hematological and biochemistry values were within normal limits for the species and age.[11,12] The affected animal was moved to an isolation tank with the same conditions described previously. The initial treatment plan included a topical tobramycin and dexamethasone solution (Tobrex®, Alcon, every 8 hours, 8 days) and nepafenac 0.1% (Nevanac®, Alcon; every 8 h, 8 days), and an intramuscular injection

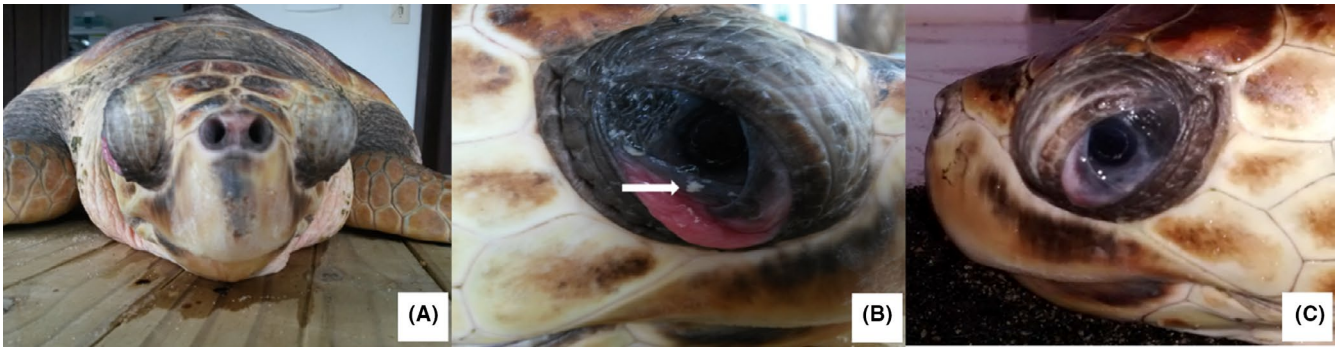

FIGURE 1 A Loggerhead turtle (*Caretta caretta*) exhibiting bilateral chemosis and conjunctival hyperemia. (A) Frontal view highlighting pronounced chemosis in the right eye. (B) Right eye with chemosis and mucoid discharge (white arrow). (C) Left eye with mild chemosis of the nictitating membrane

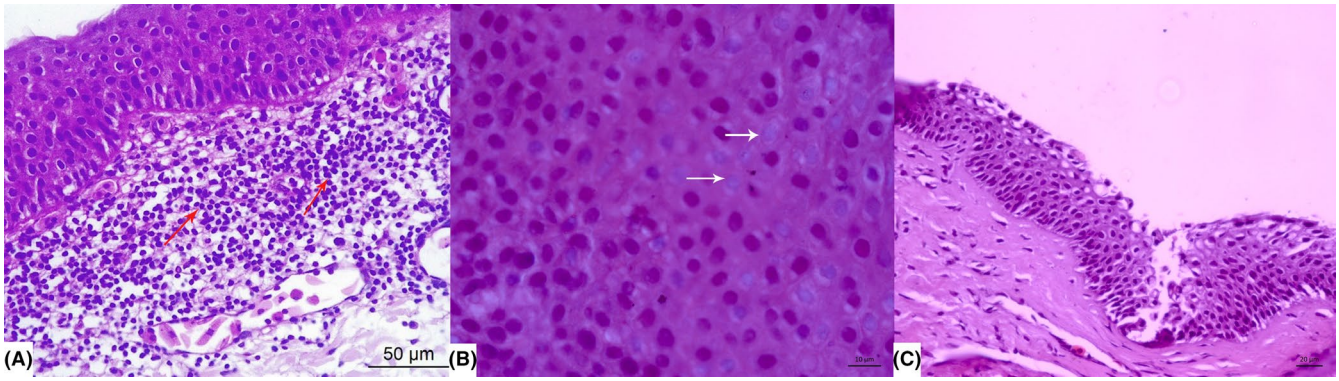

FIGURE 2 Photomicrographs of a conjunctival biopsy from the right eye of a loggerhead turtle (*Caretta caretta*) (A) Intense subepithelial inflammatory infiltration (red arrows). (H&E, 40X). (B) Note the presence of amphophilic intranuclear inclusions (white arrows) (H&E, 100X). (C) Note the reduction in the inflammatory infiltration and absence of amphophilic intranuclear inclusions (H&E–40X) after systemic treatment with vitamin A, acyclovir, and lysine

of meloxicam (Maxican® 2%, Ourofino,; 0.2 mg/kg, every 24 hours, 10 days).[13,14] Treatment resulted in a decrease in the chemosis and conjunctival hyperemia, but as there was no resolution of the ocular signs, it was decided to collect conjunctival samples for bacterial culture, cytology, and histopathology. First, bacteriology samples were collected with the animal under manual restraint, using swabs that were immediately placed in tryptose agar medium. Then, one drop of topical anesthetic (Tetracaine chlorhydrate, Anestesico®, Allergan) was administered and conjunctival cytology samples were collected using a sterile interdental brush (Interdental Brush Conical®, Oral B). These were spread on glass slides, air-dried, and stained using the Panoptic fast method.

Culture and identification of the microorganisms were performed according to routine laboratory techniques,[15] and antimicrobial susceptibility test was performed in accordance with guidelines for veterinary isolates.[16]

Finally, samples for histopathology were collected from the ventral conjunctival fornix of both eyes. Adson forceps and Westcott scissors were used to gently lift and cut, respectively, a conjunctival sample of approximately 0.3 mm^2. Two fragments were collected from each eye. The samples were embedded in paraffin, sectioned, stained with hematoxylin-eosin, and evaluated using light microscopy.

Bacteria isolated from the right eye included *Pseudomonas* sp, *Shigella* sp, and *Staphylococcus epidermidis*; *Pseudomonas* sp and *Enterobacter cloacae* were isolated from the left eye. All isolates were susceptible to polymyxin B, ciprofloxacin, neomycin, tetracycline and tobramycin, and resistant to oxacillin, chloramphenicol, and gentamicin. Exfoliative cells and hyphae were observed in the conjunctival cytology. The latter is considered part of the animal's normal flora.[17]

Conjunctival histopathology showed moderate and diffuse hyperplasia (acanthosis) associated with focal areas of hydropic degeneration. Amphophilic intranuclear inclusion bodies associated with severe, mixed subepithelial inflammatory infiltrate were seen, as well as collagenolysis, congestion, and edema. The diagnosis was subacute conjunctivitis with amphophilic intranuclear inclusions suggestive of chelonid herpesvirus [9,18] (Figure 2A,B).

3 | PCR AND SEQUENCING

DNA was extracted from conjunctival biopsy using DNeasy Blood and Tissue Kit (Qiagen). End point polymerase chain reaction (PCR) was performed for five genes associated with ChAHV5 (UL18, UL22, UL27, UL 30, and FUS3B), following standard protocols.[8] The amplification products were analyzed in agarose gel (2.0%) and stained with SYBR Safe (Thermo Fisher Scientific). Samples from ChAHV5-positive green sea turtles with known sequences were used as positive controls. The positive samples were purified in Illustra GFX PCR DNA and Gel Purification kit (GE Healthcare), and the obtained UL30 region and UL18 amplicons were sequenced via use of a BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems) and an automatic sequencer (Applied Biosystems/Perkin Elmer, model ABI Prism 310 Genetic), and aligned with similar ones available in GenBank. Fragments of DNA polymerase (UL30 region) and UL18 region were detected. Phylogenetic analysis of the 438 bp UL30 region sequence was conducted in Mega 6 based on the neighbor-joining tree method with results after 1000 bootstrap replicates. The sequence (MK953552) had 100% homology with sequences previously detected in green sea turtles from the Brazilian coast.

End point PCR analysis for the target sequences showed that the sample was positive for ChAHV5. The DNA polymerase sequence showed enough nucleotides for the analysis and construction of the phylogenetic tree and was used to characterize the viral DNA present in the sample (Figure 3).

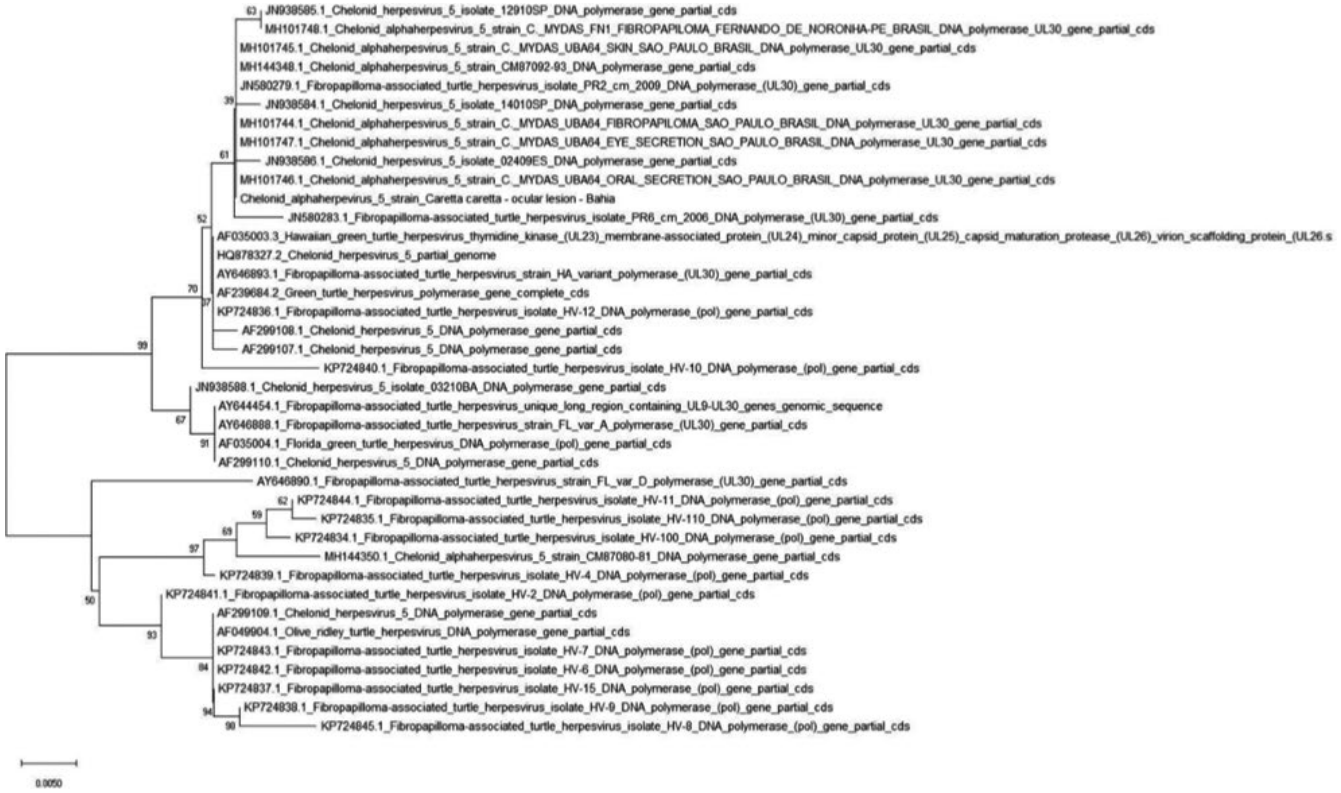

FIGURE 3 Nucleotide phylogenetic tree of DNA polymerase gene (438 bp) of the ChAHV5 in a loggerhead turtle with subacute conjunctivitis using the neighbor-joining distances method, Kimura 2-parameter model, with 1000 bootstrap replicas. SP: Brazil—São Paulo; ES—Brazil—Espírito Santo; BA—Brazil—Bahia; CE—Brazil—Ceará; PR—Brazil—Paraná; FL—Florida

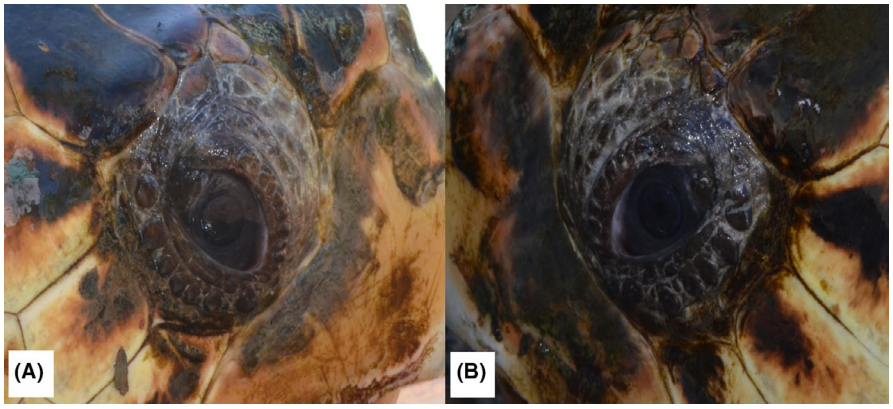

FIGURE 4 The patient seen in Figure 1 five months after the initiation of acyclovir treatment for ChHAV5 infection. Note absence of chemosis in the (A) Right eye and (B) Left eye

4 | TREATMENT AND FOLLOW-UP

Based on the PCR results and histopathological findings, the animal was treated intramuscular injections of vitamin A (A-D-E®, Pfizer; 5.000 UI/kg, 15 days, every 24 hours) and orally with L-lysine (Spring Valley Natural Foods; 20 mg/kg, every 24 hours) and acyclovir (Neo química; 80 mg/kg, every 24 hours), based on recommended doses in sea turtles.[19,20] After 25 days of treatment, during which the animal remained in isolation, there was total regression of the secretions, chemosis, conjunctival hyperemia, and globe retraction. At this time, acyclovir treatment was stopped, while treatment with L-lysine continued.

Five months after starting acyclovir treatment, another conjunctival biopsy was collected from the right eye. Histopathology showed mild epithelial hyperplasia (acanthosis) associated with diffuse hydropic degeneration, intercellular edema, and discrete inflammatory infiltrate. No amphophilic intranuclear inclusions were seen. The diagnosis was reticular degeneration associated with discrete perivasculitis (Figure 2C).

Supplementation with oral L-lysine was continued for two years, and no relapse of the secretions, chemosis, conjunctival hyperemia, and globe retraction was observed during this time, nor in the next two years of follow-up (Figure 4).

5 | DISCUSSION

There are several reasons why we did not initially suspect herpetic infection in this animal. First, the patient presented with chemosis, a sign that is seen in cases of blepharitis, keratitis, and conjunctivitis in this species, and therefore, it is not a sign that is specific for a particular diagnosis or etiological agent.[4] Second, the animal was born and bred in captivity, showed no clinical signs suggestive of immunosuppression, and water temperature and quality were monitored. In addition, other animals in the tank were clinically healthy, even though the virus is widely believed to be transmitted by water[6,7] Finally, with the exception of fibropapillomatosis, reports of herpetic disease in loggerhead turtles are infrequent.[4,21] Therefore, this atypical presentation of ChAHV5 in a sea turtle demonstrates the importance of molecular and histopathological diagnosis and appropriate treatment for ophthalmic diseases in this species.

Histopathology showed amphophilic intranuclear inclusion bodies, as well as severe inflammation, epidermal hyperplasia, and acanthosis. These findings are characteristic of viral infection in affected organs and tissues, as reported in loggerhead sea turtles[9] and freshwater turtles.[18] In our patient, the amphophilic intranuclear inclusions persisted despite the initial antibiotic and anti-inflammatory treatment, reinforcing the case for viral etiology. Herpetic infection was not suspected based on results of the complete blood count, because hematological parameters were not consistent with a state of immunosuppression in sea turtles.[6,21] It is worth mentioning that there is a considerable range of normal values for total erythrocyte and leukocyte counts in young wild turtles,[6,21,22] and therefore, results of patients' blood tests should ideally be compared to those of individuals kept under the same management conditions. In these species, immunosuppression may facilitate viral infection, especially if the animal is exposed to unfavorable environmental conditions.[2,6,21] This may explain why viral infections occur predominantly in juvenile and possibly more vulnerable sea turtles.[5] On the other hand, microbial culture results were regarded as nondiagnostic because gram-negative bacteria and hyphae are described as normal microbiota of captive loggerhead turtles,[17] while free-living sea turtles have predominantly gram-positive microbiota,[17] a difference that may be due to differences in the aquatic environment.

The diagnosis of herpesvirus was confirmed by end point PCR, which detected the presence of ChAHV5 DNA. Phylogenetic analysis revealed a sequence with 100% homology to other sequences previously detected in ocular and oral secretions, fibropapillomas, and the skin of green sea turtles from Brazil (gene codes MH101745, MH144348, MH101744, MH1017476, MH101747). Our findings demonstrate that ChAHV5 can infect other sea turtle species besides green sea turtles. Though the presence of ChAHV5 in ocular fluids without lesions or clinical presentation of herpetic conjunctivitis has been previously detected by others,[3] our results demonstrate that it can also cause epithelial lysis and inflammation of the conjunctiva.

ChAHV is an important cause of morbidity in sea turtles. Studies suggest that different strains of herpesvirus cause different lesions.[9,18] The virus can cause latent infection or a disease that ranges from mild to severe [2,8,9,21,23] with the latter requiring intensive care and long quarantine of affected animals.[2,6] The virus may also cause fatal disease in loggerhead turtles as the proliferating fibropapilloma masses obscure the eye, causing blindness and inability to feed.[2,4-6] The effects of visceral nodules on organ function, and decreased mobility due to proliferation on the limbs, are additional causes of mortality.[2,3,6,7]

Treatments described for herpesvirus in reptiles include antivirals and supplements that aid in restoring the immune system. We therefore prescribed vitamin A supplementation and acyclovir at a dose (80 mg/kg) reported for sea turtles with herpesvirus.[20] In another Chelonian species (*Testuno* sp), acyclovir and ganciclovir were used to manage herpetic stomatitis.[23] L-lysine was added as it may interfere with viral replication, even though the efficacy of this oral supplementation is controversial.[24] The 0.2 mg/kg dose of meloxicam that we used was based on recommendations for reptiles, rather than on species-specific recommendations.[13,14] In retrospect, the dose may have been too low, and it is possible that the therapeutic anti-inflammatory effect was achieved by the topical drug that we used.

In conclusion, chemosis in free-living and captive sea turtles may be a clinical manifestation of ChAHV5 disease. Diagnosis can be confirmed by histopathology and PCR, and acyclovir and L-lysine treatment may be efficacious.

ORCID

Arianne P. Oriá https://orcid.org/0000-0001-5093-6841
Ron Ofri https://orcid.org/0000-0002-3825-3113

REFERENCES

1. Casale P, Tucker AD. Caretta caretta, Loggerhead Turtle. The IUCN Red List of Threatened Species. 2017;1-18.
2. Abreu-Grobois FA, Briseño-Dueñas R, Márquez-Millán L, et al.Proceedings of the 18th International Sea Turtle Symposium. 2000; 32:64-150.
3. Monezia TA, Mehnert DU, Moura EMM, et al. Chelonid herpesvirus 5 in secretions and tumor tissues from green turtles (*Chelonia mydas*) from Southeastern Brazil: a ten-year study. *Vet Microbiol*. 2016;186:150-156.
4. Işler CT, Altuğ M, Cantekİn Z, et al. Evaluation of the eye diseases seen in Loggerhead Sea turtle (*Caretta caretta*). *Rev Med Vet*. 2014;165:258-262.
5. Lawrance MF, Mansfield KL, Sutton E, et al. Molecular evolution of fibropapilloma-associated herpesviruses infecting juvenile green and loggerhead sea turtles. *Virology*. 2018;521:190-197.
6. Jones K, Ariel E, Burgess G, et al. A review of fibropapillomatosis in Green turtles (*Chelonia mydas*). *Vet J*. 2016;212:48-57.
7. Ariel E. Viruses in reptiles. *Vet Res*. 2011;42:2-12.

8. Alfaro-Núñez A, Bojesen AM, Bertelsen MF, et al. Further evidence of Chelonid herpesvirus 5 (ChAHV5) latency: high levels of ChAHV5 DNA detected in clinically healthy marine turtles. *Peer J*. 2016;4:e2274.
9. Stacy BA, Wellehan JFX, Foley AM, et al. Two herpesviruses associated with disease in wild Atlantic Loggerhead Sea Turtles (*Caretta caretta*). *Vet Microbiol*. 2008;126:63-73.
10. United States Department of the Interior Fish and Wildlife Service. Standard permit conditions for care and maintenance of captive sea turtle. 2013;1–16.
11. Reséndiz E, Fernández-Sanz H, Barrientos-Torres DS, Lara-Uc MM. Clinical pathology and health reference values for loggerhead sea turtles (*Caretta caretta*) and olive ridley turtles (*Lepidochelys olivacea*) in the Gulf of Ulloa, Baja California Sur, Mexico. *Comp Clin Pathol*. 2019;28:1637-1650.
12. Yang T, Haas HL, Patel S, Smolowitz R, James MC, Williard AS. Blood biochemistry and haematology of migrating loggerhead turtles (*Caretta caretta*) in the Northwest Atlantic: reference intervals and intra-population comparisons. *Conserv Physiol*. 2019;7:coy079.
13. Norton TM. Chelonian emergency and critical care. *Semin Avian Exot Pet Med*. 2005;14:106-130.
14. Hernandez-Divers SJ. *Meloxicam and Reptiles – A Practical Approach to Analgesia. Small Animal and Exotics*, 2006: 1636-1637. Orlando, FL: Proceedings of the North American Veterinary Conference.
15. Procop GW, Church DL, Hall GS, et al. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 7th edn. Philadelphia, PA: Wolters Kluwer Health; 2016:1936.
16. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2016;37(1):i-248.
17. Cardoso-Brito V, Raposo ACS, Pires TT, et al. Conjunctival bacterial flora and antimicrobial susceptibility of captive and free-living sea turtles in Brazil. *Vet Ophthalmol*. 2019;22:246-255.
18. Jungwirth N, Bodewes R, Osterhaus ADME, et al. First report of a new alphaherpesvirus in a Freshwater Turtle (*Pseudemys concinna* concinna) kept in Germany. *Vet Microbiol*. 2014;170:403-407.
19. Harkewicz KA. Dermatology of reptiles: a clinical approach to diagnosis and treatment. *Vet Clin North Am Exot Anim Pract*. 2001;4:441-461.
20. Page-Karjian A, Norton TM, Krimer P, et al. Factors influencing survivorship of rehabilitating Green Sea Turtles (*Chelonia mydas*) with fibropapillomatosis. *J Zoo Wildlife Med*. 2014;45:507-519.
21. Orós J, Montesdeoca N, Camacho M, et al. Causes of stranding and mortality, and final disposition of Loggerhead Sea Turtles (*Caretta caretta*) admitted to a wildlife rehabilitation center in Gran Canaria Island, Spain (1998–2014): a long-term retrospective study. *PLoS One*. 2016;11:1-14.
22. Manire CA, Norton TM, Stacy BA, et al. *Sea Turtle Health & Rehabilitation*. Plantation, FL: J Ross Publishing Inc; 2017:1010.
23. Marschang RE, Gravendycankd M, Kaleta EF. Herpesviruses in tortoises: Investigations into virus isolation and the treatment of viral stomatitis in *Testudo hermanni* and *T. graeca*. *J Vet Med*. 1997;44:385-394.
24. Gould D. Feline herpesvirus: ocular manifestations, diagnosis and treatment options. *J Feline Med Surg*. 2011;13:333-346.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Oriá AP, Silva DN, Raposo AC, et al. Atypical ocular Chelonoid herpesvirus manifestations in a captive Loggerhead turtle (*Caretta caretta*). *Vet Ophthalmol*. 2020;00:1–6. https://doi.org/10.1111/vop.12837