Identification of Fibropapillomatosis in Green Sea Turtles (*Chelonia mydas*) on the Texas Coast

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Abstract: Nine green sea turtles, *Chelonia mydas*, were presented to two rehabilitation facilities on the Texas coast with cutaneous growths consistent with fibropapillomatosis. Complete blood counts, radiographs, and computed tomography were performed for further evaluation. No evidence of internal tumors was present using either imaging modality. Treatment included surgical excision of the cutaneous tumors. Histopathologic analysis and polymerase chain reaction (PCR) were performed with the tissue samples collected. Histopathology revealed characteristic inclusions in only three (33%) individuals, and PCR results for fibropapilloma-associated turtle herpesvirus were positive for eight (89%) of nine individuals submitted. To our knowledge, this is the first report of fibropapillomatosis in a green sea turtle on the Texas coast.

Key Words: *Chelonia mydas*, fibropapilloma, fibropapillomatosis, fibropapilloma-associated turtle herpesvirus, tumor, green turtle.

Case Report

Nine green sea turtles, *Chelonia mydas*, were presented to two rehabilitation facilities (Animal Rehabilitation Keep, The University of Texas at Austin, Port Aransas, TX and Sea Turtles Inc., South Padre, TX) on the Texas coast during May, June, and August 2010 with cutaneous growths consistent with fibropapillomatosis. All individuals weighed between 5 and 40 kg (mean = 13.7 kg, SD = 10.58703) and were considered subadults based on body weight and carapace measurements. One of the individuals was dead upon arrival, one individual was in poor body condition (body condition score 2/5), and the remaining individuals were in fair body condition (3/5). Cutaneous masses were noted on the eyes, head, neck, flippers, tail, and cutaneous–carapacial junction and measured from <1 to >10 cm in diameter (Figs. 1–3).

Complete blood counts and biochemistry testing were performed on admission to the rehabilitation facilities. Radiographs and computed tomography also were performed to determine whether internal masses were present and to determine whether surgical intervention should be pursued to remove the cutaneous masses. No radiographic evidence of internal masses was seen in any of the turtles. Computed tomography was performed to further evaluate the lungs in seven of the eight green sea turtles due to its increased sensitivity; no pulmonary or internal masses were noted. In addition, a full necropsy was performed on the deceased individual, with no evidence of internal masses found.

Biopsy samples from each of the eight live green sea turtles were submitted for histopathology and molecular diagnostic testing by polymerase chain reaction (PCR) for fibropapilloma-associated turtle herpesvirus (FPTHV). In addition, samples from the deceased turtle also were submitted for histopathology and PCR testing. PCR results for FPTHV were positive for eight (89%) of nine individuals submitted. The lesions from all animals were characterized histologically by varying degrees of regional dermal fibrous connective tissue proliferation and mild-to-moderate papillary hyperplasia of the overlying epidermis. All lesions exhibited vacuolar (hydropic) degeneration of the epithelial cells, with changes most severe in the basilar and spinosum layers of the epidermis. Nuclei in the regions of papillary hyperplasia exhibited chromatin clumping with occasional chromatin marginalization. Rare individual keratinocytes in three (33%) of the turtles contained central intranuclear eosinophilic, round-to-ovoid inclusion bodies. Moderate lymphoplasmacytic and histiocytic inflammation extended throughout the proliferative dermal connective tissue, with

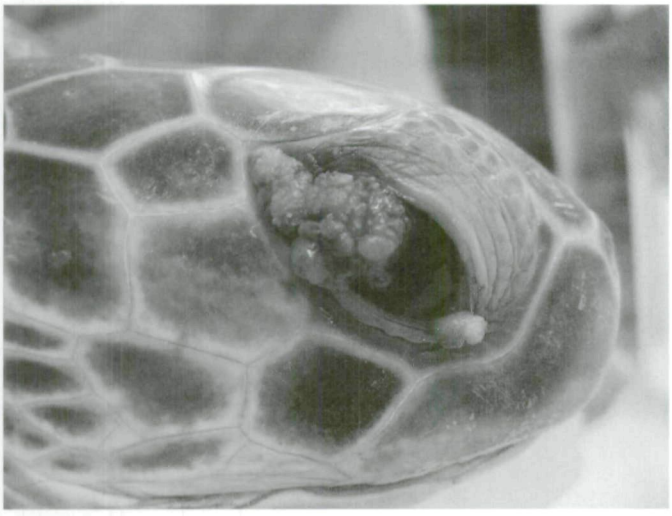

Figure 1. Fibropapilloma on the right eye.

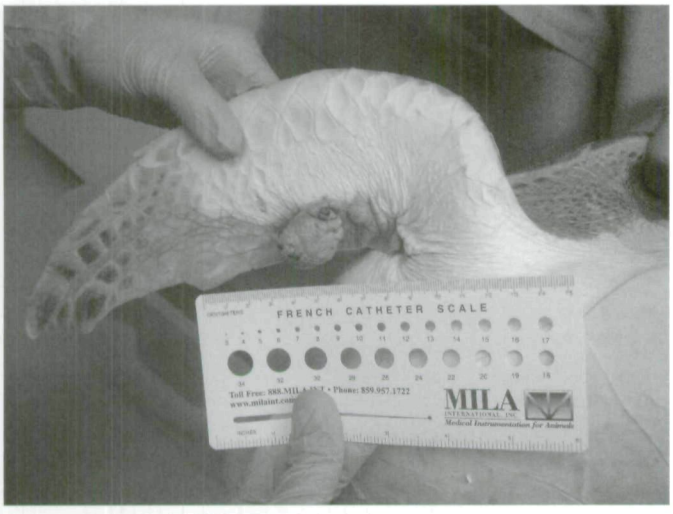

Figure 2. Fibropapilloma on the caudoventral aspect of the right flipper.

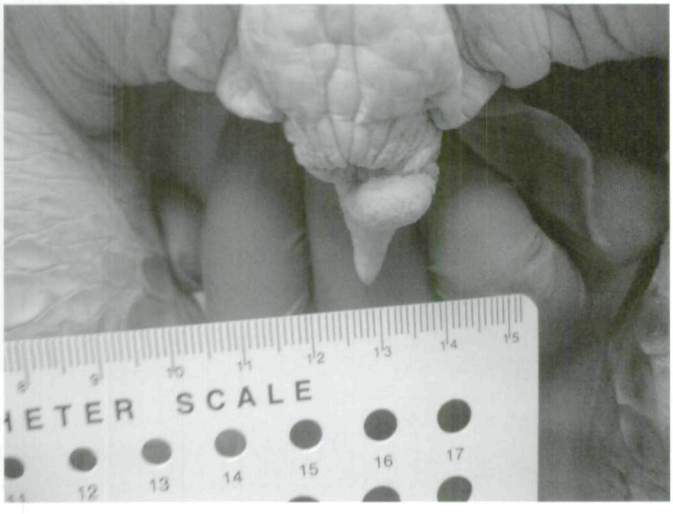

Figure 3. Fibropapilloma in the pericloacal region.

a primarily perivascular pattern. Denser perivascular lymphoplasmacytic infiltrates were present at the periphery of the proliferating dermal connective tissue. Both the proliferative and degenerative epithelial changes were mild in two of the turtles, and one of these turtles tested negative on PCR.

The eight turtles with fibropapillomatosis were taken to surgery for removal of the masses. Seven recovered without incident, and one individual died during recovery from anesthesia. The turtle that died had the largest and most severe distribution of tumors, which may have contributed to its postoperative death. The remaining seven turtles healed without complication and were released between 8 and 12 wk after surgery.

Discussion

Fibropapillomatosis of sea turtles is a neoplastic disease caused by FPTHV. The disease is primarily seen in green sea turtles, but it also has been identified in the loggerhead, *Caretta caretta*; olive ridley, *Lepidochelys olivacea*; hawksbill, *Eretmochelys imbricata*; flatback, *Natator depressus*; and leatherback, *Dermochelys coriacea* (Herbst, 1994; Huerta *et al.*, 2002). Green sea turtle fibropapillomatosis was first described in 1938 in the Florida Keys (Lucke, 1938; Smith and Coates, 1938). Few reports of disease were identified between that time and the 1980s, but the prevalence rose soon after in Florida and Hawaii (Herbst, 1994). Fibropapillomatosis has since been identified in Puerto Rico, the Virgin Islands, Barbados, Costa Rica, Venezuela, Colombia, Panama, Belize, Hawaii, Indonesia, and Australia (Jacobson *et al.*, 1991; Lackovich *et al.*, 1999). Increased prevalence in the disease has been hypothesized to be the result of the recent introduction of FPTHV into naive marine animal populations (Greenblatt *et al.*, 2005). A second hypothesis suggests that the virus was already established in the population and environmental cofactors including habitats in proximity to agricultural and urban development has contributed to an increased prevalence (Greenblatt *et al.*, 2005; Van Houtan *et al.*, 2010). Green sea turtles have been closely monitored in Texas through the Sea Turtle Stranding and Salvage Network and directed capture netting studies. Before this report, no documented cases of fibropapillomatosis had been identified on the Texas coast (Shaver, 2000).

The green sea turtles in this report were of similar size (curved carapace length: mean = 46.9 cm; SD = 10.281; range, 36.3–70.1 cm) and age class (subadult) as seen in other green sea turtle populations affected by fibropaillomatosis (Foley *et al.*, 2005). Reports from Florida, Hawaii, Brazil, Indonesia, and Australia identify subadult green sea turtles as the most susceptible to fibropapillomatosis (Adnyana *et al.*, 1997; Work and Balazs, 1999; Foley *et al.*, 2005; Flint *et al.*, 2009; Guimaraes dos Santos *et al.*, 2010). The reasons for this association are unclear, although multiple hypotheses have been proposed. One hypothesis proposes that subadult animals become infected when they migrate from the open ocean to neritic (nearshore) habitats as part of their normal maturation. This idea was supported by evaluations of viral variants by Ene *et al.* (2005). Pollution due to industrial runoff is another possible explanation for the decreased immune function and subsequent increased prevalence of infection with FPTHV (Foley *et al.*, 2005;

Guimaraes dos Santos *et al.*, 2010). Other theories include heavy parasite loads, immunosuppression, marine toxins, and bacteremia (Work *et al.*, 2001, 2003, 2005).

The mode of transmission of FPTHV is still unknown, but marine leeches and other marine organisms have been proposed as possible vectors for the disease (Lu *et al.*, 2000; Greenblatt *et al.*, 2004). In addition, immunosuppression due to various environmental factors also is thought to play a role in transmission of the virus (Adnyana *et al.*, 1997; Foley *et al.*, 2005; Flint *et al.*, 2009). An experimental transmission study was performed using cell-free tumor extracts (or homogenates) in green sea turtles that demonstrated transmissibility of the virus (Herbst *et al.*, 1995). However, to date, Koch's postulates have not been fulfilled to demonstrate a relationship between FPTHV and fibropapillomas due to the virus being refractory to passage in cell cultures (Lachovich *et al.*, 1999).

Evaluation and scoring of fibropapillomas can aid in the description of the tumor size and severity of disease, and ultimately may affect the course of therapy for individual turtles. Scoring systems have been established, but a standard or universal scoring system has not yet been adopted. The turtles in this report had tumor scores defined based on previous studies (Balazs, 1991). Individual tumors were classified into four basic categories: <1 cm (size A), 1–4 cm (size B), 4–10 cm (size C), and >10 cm (size D). The turtles in this report had tumors that were similar in location and size to those from previous reports for this species. Interestingly, turtles in Hawaii are noted to have tumors in the oral cavity 50% of the time, which is a higher percentage than that noted in other parts of the world where fibropapillomatosis is reported (Work *et al.*, 2004). The difference may be due to genomic variation in FPTHV from different geographic regions (Greenblatt *et al.*, 2005). Cutaneous tumor location is often concentrated in the inguinal and axillary regions, but they are also found around the neck, tail, conjunctiva, and cornea. Internal fibropapillomas may develop in multiple visceral sites, including the lungs, liver, kidney, and gastrointestinal tract. Only cutaneous tumors in the locations listed above were seen in the green sea turtles in this report. No evidence of oral or internal fibropapillomas was seen on either necropsy or diagnostic imaging of these individuals.

Diagnosis of fibropapillomatosis can be accomplished by several methods, but the standard is molecular testing by PCR. Various blood parameters have been evaluated in turtles with fibropapillomatosis. Hematologic parameters examined in FPTHV-positive green sea turtles from Hawaii showed a normal heterophil and white cell count, an increase in monocytes, and a decrease in eosinophils and lymphocytes (Work and Balazs, 1999). Biochemical parameters examined in captive and wild turtles with fibropapillomatosis in another study showed a correlation between FPTHV-infected turtles and elevated alkaline phosphatase and significantly lower levels of lactate compared with turtles without tumors (Swimmer, 2000). The hematologic and biochemical parameters of the turtles from this report were all within reference ranges for the species (Mader, 2006). Despite this fact, complete and thorough clinicopathologic evaluation of reference ranges for green sea turtles is lacking and further investigation is needed.

Evaluation and diagnosis of internal tumors can be performed by radiography, laparoscopy, computed tomography, and magnetic resonance imaging (Croft *et al.*, 2004). Radiography is a minimally invasive method for evaluating internal tumor growth. Radiographs were performed on the eight live green sea turtles in this report, with no internal tumors detected. Although radiography is an adequate screening tool, tumors may not be identified due to the decreased sensitivity for small tumors. In addition, tumors may be obscured by the superimposition of the carapace and plastron. The difficulty in identifying tumors of this size prompted us to pursue computed tomography. No evidence of tumors was noted in any of the green sea turtles evaluated with computed tomography. Magnetic resonance imaging and laparoscopy are two additional methods that may be used to further screen for internal tumors, but these methods were not pursued due to lack of equipment availability. Histopathology was performed on all samples, and characteristic inclusions were seen only rarely in three individuals. Histopathology may or may not reveal inclusions based on the distribution of viral particles (Jacobson *et al.*, 1991). Thus, multiple tumors on an individual turtle should be collected and examined to determine whether inclusions are present. Molecular diagnostics also were performed via PCR testing for FPTHV. Eight (89%) of nine individuals were positive for FPTHV. The one negative result could be explained by the small sample size or collection of the sample outside the border of the tumor. The former explanation is more likely because all tissues were biopsied in the bulk mass of the excised tumors. Further testing for viral genomic variation was not performed at this time due to financial constraints.

Treatment for fibropapillomatosis primarily relies upon surgical removal of the masses by using a variety of techniques (Mader, 2006). Radiocautery and CO_2 laser therapy are the most frequently used methods for removing tumors. Prognosis for turtles with fibropapillomatosis depends on multiple factors, including size, location, and invasive nature of the tumors. The current recommendation for turtles with internal fibropapillomas is euthanasia due to the poor prognosis for long-term recovery (Mader, 2006). Tumors on the eye may be life threatening if they invade the cornea and cannot be removed without enucleation. Despite this fact, turtles do well after release with enucleation of one eye (Tristan, unpublished data).

The eight turtles with fibropapillomatosis were taken to surgery, and the masses were excised with radiocautery. One individual died during recovery from anesthesia. This individual had the largest and most severe distribution of tumors, which may have contributed to its postoperative death. The remaining seven green sea turtles recovered from anesthesia, healed without complication, and were released between 8 and 12 wk after surgery. A satellite transmitter was placed on the largest individual, and monitoring continues as of the writing of this article. In addition, monitoring for further cases of fibropapillomatosis is currently underway on the Texas coast. This case report represents the first documentation of fibropapillomatosis in green sea turtles on the Texas coast.

Acknowledgments: We thank our respective institutions for support of this work. Jennifer Shelby Walker; Amanda Terry, RVT; Shauna Ertolacci; Adrienne McCracken; Colt Snapp; and Katie St. Clair assisted with documentation management, necropsy, collection and shipment of specimens,

and the care of the animals. A.L. was the holder of the federal and state scientific permits that authorized capture of green sea turtles in the Lower Laguna Madre.

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