

Pathology of Oropharyngeal Fibropapillomatosis in Green Turtles *Chelonia mydas*

A. ALONSO AGUIRRE*

Wildlife Trust, Columbia University,
61 Route 9 West,
Palisades, New York 10964-8000, USA

GEORGE H. BALAZS

National Marine Fisheries Service,
2570 Dole Street,
Honolulu, Hawaii 96822-2396, USA

TERRY R. SPRAKER

State Veterinary Diagnostic Laboratory,
Colorado State University,
Fort Collins, Colorado 80523, USA

SHAWN K. K. MURAKAWA

National Marine Fisheries Service,
2570 Dole Street,
Honolulu, Hawaii 96822-2396, USA

B. ZIMMERMAN

Wildlife Health International,
Post Office Box 1522,
Fort Collins, Colorado 80522, USA

Abstract.—Complete gross and histopathologic examinations of the oral cavity, tongue, pharynx, larynx, and glottis were performed in five Hawaiian green turtles *Chelonia mydas* with fibropapillomatosis. These examinations demonstrated that the oropharyngeal fibropapillomas were similar to characteristic external fibropapillomas previously described for green turtles. The size, appearance, and anatomic site of the tumors confirmed that these turtles presented total or partial occlusion of the nasopharynx, glottis, larynx, and adjacent tissues. The fibropapillomas of the oropharynx were considered locally invasive and severely modified the morphophysiology of respiration and feeding in these turtles. To the best of our knowledge, this is the first report of oropharyngeal fibropapillomatosis in sea turtles.

Marine turtle fibropapillomatosis (FP) is one of the most important health problems affecting free-ranging sea turtles (George 1997). Although reported since the late 1930s in Florida (Smith and Coates 1938) and since 1958 in the Hawaiian Islands (Balazs and Pooley 1991), the disease has

recently reached epizootic proportions. It has primarily affected green turtles *Chelonia mydas* in many pantropical regions of the world; however, it has been confirmed histopathologically in five other species. The potential interactions and epidemiological links of the hypothesized causes of FP have been the subject of discussion. The molecular identification of one or more herpesviruses, a papilloma-like virus, and a retrovirus in tumors from different species around the world has complicated the task of determining the primary etiology of tumor formation. Other cofactors may play a role in the etiology (Herbst 1994; Quackenbush et al. 1998; Landsberg et al. 1999; Aguirre et al. 2002).

These debilitating and disfiguring tumors can measure from 1 mm to 40 cm in diameter and have been reported in keratinized tissues including the skin, plastron, and carapace. The tumors are most prominent on the axillae, inguinal region, neck, cloaca, and tail, as well as between the scales and scutes and in the eyes and conjunctiva. Ocular fibropapillomas have been described as locally invasive and associated with severe blindness, secondary panophthalmitis, destruction of the globe,

* Corresponding author: aguirre@wildlifetrust.org

Received April 23, 2002; accepted September 20, 2002



FIGURE 1.—The oropharyngeal region of a green turtle stranded in the Hawaiian Islands. Pedunculated, cauliflower-like fibropapillomas encircle the glottis (reflected laterally by forceps) and obstruct adjacent laryngeal tissues.

and debilitation (Brooks et al. 1994). Tumors have been reported in most internal organs. Visceral fibromas are known to cause cardiac and respiratory disease, hydronephrosis, and gastrointestinal obstruction (Herbst 1994). The purpose of the present study was to describe the epidemiology and histopathology of oropharyngeal FP.

Methods

Five stranded green turtles with oropharyngeal fibropapillomatosis (OFP) were recovered live in the Hawaiian Islands for detailed pathologic examination. These turtles were selected for pathologic study based on their inability to survive in the wild. Four of them were transported to the National Marine Fisheries Service (NMFS) Honolulu Laboratory, where they were euthanized with

a lethal intraperitoneal injection of Beuthanasia-D Special Solution (Schering-Plough Animal Health, Kenilworth, New Jersey); the remaining one, which was found at Haleiwa on the island of Oahu, was maintained in a tank at the NMFS Kewalo Basin Research Facility for several days for diagnostic evaluation prior to its death. Necropsies were performed following a preestablished protocol (Wolke and George 1981). Special examination of the buccal cavity, nares, tongue, soft and hard palates, pharynx, larynx, glottis, and adjacent tissues was performed, and the number of fibropapillomas was recorded. Standard techniques were followed for the histopathologic evaluation of specimens (Luna 1968). Briefly, the tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6- μ m, and stained with

TABLE 1.—Characteristics of five green turtles from Hawaii and the oropharyngeal, glottal, and laryngeal fibropapillomas (OFP) found in them and subjected to histopathologic examination. The fibropapilloma severity score (FPS) is defined as follows: 3, heavily affected; 2, moderately affected; and 1, lightly affected. Turtles without external evidence of fibropapillomas were given a score of 0. Anatomic site influenced FPS when vision and ability to breathe or feed were considered impaired (Aguirre et al. 1998; Balazs and Pooley 1991; Work and Balazs 1999).

Stranding location	Identification code	Sex ^a	Straight carapace length (cm)	Weight (kg)	FPS	Number of external tumors
Waikiki, Oahu	A	F	50.9	16.8	3	56
Waimea Beach, Oahu	B	U	52.0	15.5	3	47
Haleiwa, Oahu	C	F	49.9	12.7	2	21
Kaneohe Bay, Oahu	D	U	48.7	12.7	3	14
Puamana, Maui	E	M	46.0	9.5	2	24

^a Female (F), male (M), or undetermined (U).

hematoxylin and eosin, periodic acid Schiff, or Masson stain.

Results

The descriptive and pathological characteristics of five green turtles with large white multilobulated masses involving the laryngeal and glottal regions were analyzed (Table 1). Turtles A–C presented papillary growths causing partial or total obstruction of the glottis, epiglottis, pharynx, larynx, and esophagus (Figure 1). In some instances, fibropapillomas were protruding from the laryngeal folds through the glottis and into the lumen of the larynx (Figure 2). In turtles D and E,

growths were identified on the tongue and palates that were obstructing the passage of food to the esophagus.

A total of 27 oropharyngeal, glottal, and laryngeal tumors were examined. Histopathologically, all tumors in this study were diagnosed as fibropapillomas (Figure 3). The histopathologic characteristics of these tumors are summarized in Table 2. These fibropapillomas had more keratin pearls than the characteristic external fibropapillomas. Lesions were characterized by moderate acanthosis and mild hyperkeratosis. Six tumors from two turtles presented several small focal areas of ballooning degeneration of epithelial cells and intra-



FIGURE 2.—Transversal cuts demonstrating (from left to right) a normal larynx, a fibropapilloma on the lateral aspect of a larynx with constriction, and partial obliteration of the larynx and compression caused by a tumor.

TABLE 1.—Extended.

Stranding location	Number and description of OFPs	Anatomic site	Mean size (mm)	Gross observations
Waikiki, Oahu	1, pedunculated	Tongue, larynx	20 × 25	Oropharynx obstruction
Waimea Beach, Oahu	5, papillary	Larynx, glottis	5 × 10	Larynx partial obstruction
	3, pedunculated	Pharynx, larynx	15 × 30	Total obstruction
Haleiwa, Oahu	1, fibromatous	Pharynx, larynx	5 × 25	Partial obstruction
	6, papillary	Tongue, larynx	5 × 20	Oropharynx obstruction
	4, fibromatous	Pharynx	20 × 30	Necrotic; total obstruction
Kaneohe Bay, Oahu	3, fibromatous	Palates, tongue	25 × 30	Necrotic; palate obliteration
Puamana, Maui	4, papillary	Palates, tongue	10 × 30	Palate obliteration

nuclear inclusion bodies (Figure 4). The fibroblastic superficial dermal layer was composed of fusiform cells with elongated nuclei containing fibrillar cytoplasm. Inflammatory cells, primarily lymphocytes, surrounded the vessels within these lesions. Multiple glottal papillary growths were composed of a core of dense connective tissue with proliferative lesions primarily made up of mesenchymal or fibroblastic cells.

Necrosis and ulceration with degrading keratin, necrotic epithelium, cellular debris, and bacterial proliferation were evident in four buccal fibropapillomas of turtle C and three glottal fibromas of turtle D. Small pyogranulomas and spirorichid

trematode ova engulfed by multinucleated giant cells were present in all oropharyngeal fibropapillomas. No other associated organisms were identified.

Discussion

The histopathology of external fibropapillomas has been described previously for green turtles in Florida (Jacobson et al. 1989) and the Hawaiian Islands (Aguirre et al. 1994). The usual outcome of most affected turtles in Hawaii is debilitation over a protracted period, followed by death. The disease is known to interfere with the hydrodynamic and buoyancy features of turtles, thereby

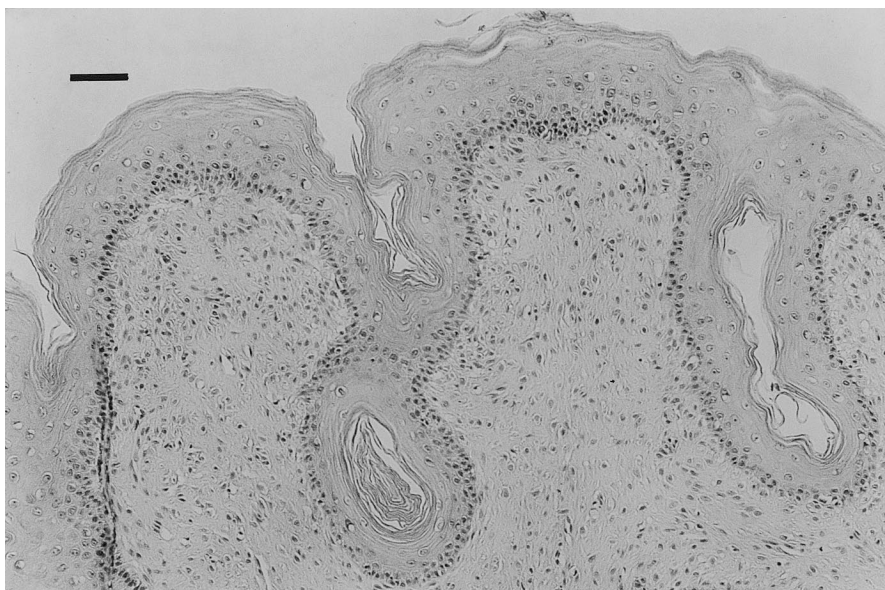


FIGURE 3.—Light micrograph of an oropharyngeal fibropapilloma tissue section from the glottis stained with hematoxylin and eosin. Observe the proliferating mesoderm with minimal hyperkeratosis and acanthosis. Scale bar = 33 μ m.

TABLE 2.—Histopathologic changes of 27 oropharyngeal, glottal, and laryngeal fibropapillomas of five green turtles.

Histopathologic change	Number (%)
Epithelium	
Papillary pattern	19 (70)
Linear pattern	8 (30)
Acanthosis	25 (93)
Hyperkeratosis	27 (100)
Pseudoepitheliomatous hyperplasia	24 (89)
Intercellular edema	17 (63)
Intracellular edema	9 (33)
Ballooning degeneration	6 (22)
Cytoplasmic vacuolar degeneration	5 (18)
Individual cell necrosis	3 (11)
Necrosis (full depth) with underlying inflammation	7 (26)
Lymphocytic infiltration, stratum basale	21 (78)
Margination of chromatin with intranuclear inclusions	6 (22)
Dermis and fibroblastic portion	
Fibroblastic proliferation patterns	
Sheets	17 (63)
Interweaving bundles	10 (37)
Cellularity of tumor	
Low	7 (26)
Moderate	20 (74)
Foci of lymphocytic inflammation	21 (78)
Foci of necrosis	7 (26)
Granulomas containing parasitic ova	27 (100)
Keratin pearls	27 (100)
Subdermal tissue	
Deep fibroblastic reaction	10 (37)
Vessels cuffed with lymphocytes	27 (100)
Granulomas containing parasitic ova	27 (100)

affecting their ability to swim. If the tumors are located around the eyes and mouth, the turtles present reduced vision or complete blindness, disorientation, and physical obstruction of basic functions such as feeding and breathing. In addition, FP may drastically reduce the capacity for predator avoidance and increase the susceptibility of affected individuals to stranding, thus threatening their survival (Herbst 1994).

Green turtles are mouth breathers that use their nasal passages mainly for chemoreception. The epithelium of the normal buccal cavity is smooth and covered by a mucous membrane composed of a squamous, nonkeratinized epithelium and lamina propria of a connective tissue, but it lacks a muscularis mucosa. The tongue is fleshy, short, broad in the base, and firmly attached to the floor of the mouth along its entire length and is covered by a compound, partially keratinized squamous epithelium pierced by ducts of mucous glands (Luppa 1977; Rainey 1981). The fibropapillomas de-

scribed herein in the oropharynx and adjacent tissues could affect the swallowing and respiratory dynamics of turtles. A periodic ejection of water through the nares accompanies the deglutition process, including the acquisition of food suspended in water and the propulsion of a bolus from mouth to stomach by expansion of the pharyngeal cavity (Seymour 1982; White 1994). A total obstruction of the posterior nares and occlusion of the glottis by a fibroma disrupts this process. Furthermore, an alteration of the pharyngeal and esophageal pressures supporting the propulsion of food into the stomach may occur. The obstruction of the glottis may disrupt the normal respiratory cycle of green turtles, which is characterized by a forceful expiration (blow) followed by a rapid inspiration of air (Walker 1959).

Additional data will be necessary to determine the anatomical predilection of FP for the oropharyngeal, glottal, and laryngeal tissues in Hawaiian green turtles as compared with those in Florida and other parts of the world. Inspection of hundreds of live and euthanized green turtles from the Indian River Lagoon, Florida, showed no evidence of oropharyngeal fibropapillomas except for some minor involvement of the upper maxilla rhamphotheca external to the oral cavity (L. M. Ehrhart, University of Central Florida–Orlando, personal communication; E. R. Jacobson, University of Florida–Gainesville, personal communication; B. A. Schroeder, National Marine Fisheries Service, personal communication). Green and loggerhead turtles *Caretta caretta* are routinely examined for the presence of OFP in Australia, and no evidence of oropharyngeal involvement has been reported except for a small, 1.5-cm tumor near the hard palate of a green turtle. Continued monitoring of these populations is recommended.

Retrospective analysis of data for the Hawaiian green turtle population indicates that fibropapillomas commonly occur in the oropharynx, glottis, and larynx. Between 1991 and 1995, 561 green turtles were captured alive, closely examined, and released in Kaneohe Bay. Over 42% of these turtles (236/561) presented FP, and 40% (94/236) of those demonstrated OFP (G. H. Balazs, A. A. Aguirre, and S. K. K. Murakawa, unpublished data).

No scientific explanation exists for the differences between the Hawaiian and other populations of green turtles. Routes of exposure and transmission at different anatomic sites may differ among populations, depending on their geographic distribution. A plausible factor is the greater number

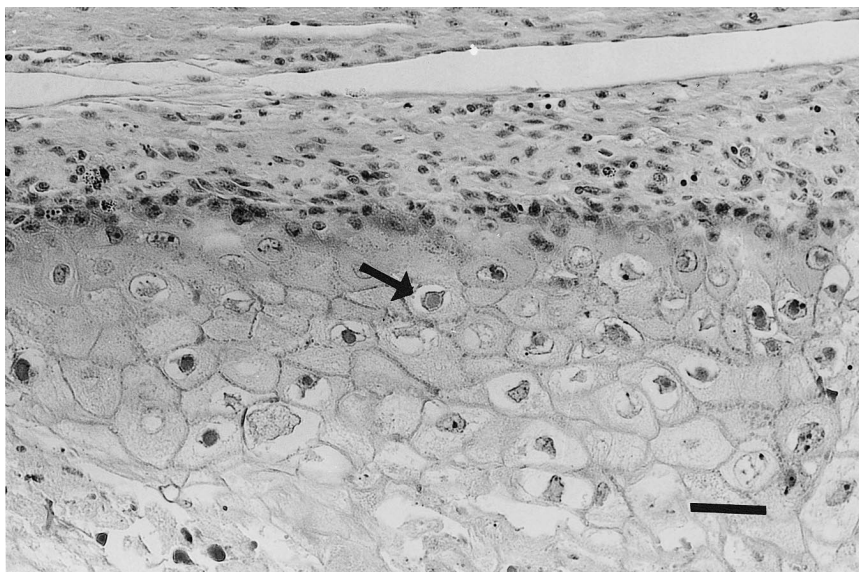


FIGURE 4.—Light micrograph of an oropharyngeal fibropapilloma tissue section from the glottis stained with hematoxylin and eosin. The epidermis demonstrates small focal areas of ballooning degeneration of epithelial cells and intranuclear inclusion bodies (arrow). Scale bar = 66 μ m.

of ectoparasites, including the marine leech *Ozobranchius branchiatus* and *Hyachelia tortugae* amphipods in the oral cavity of Hawaiian green turtles. Damage to oral tissues caused by these organisms may serve to promote infection by the etiologic agent of FP (Aguirre et al. 1994, 1998; Dailey and Morris 1995).

The five turtles in this report were considered to have advanced stages of the disease; the fibropapillomas of the oropharynx were considered locally invasive and of sufficient size to have adversely affected their respiration or feeding. Monitoring of oral tumors is recommended when evaluating the clinical condition of wild individuals. Clinical evaluations of turtles in rehabilitation should also include careful oropharyngeal examinations. Studies of the progression of the disease are needed to establish the impacts of OFP on sea turtle populations in Hawaii and Australia and whether these same impacts occur in Florida and other parts of the world.

Acknowledgments

We acknowledge the assistance of R. Morris, Makai Animal Clinic, Kailua, Hawaii, S. E. Eames, and D. M. Parker. G. Antonelis, L. M. Ehrhart, R. H. George, W. G. Gilmartin, S. Pooley, J. Wetherall, and G. C. Whittow reviewed previous drafts of this manuscript. This research was sponsored in part under contract 40JJNF50062 from

the NMFS Southwest Fisheries Science Center, Honolulu Laboratory, Hawaii.

References

- Aguirre, A. A., G. H. Balazs, B. Zimmerman, and T. R. Spraker. 1994. Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomas. *Journal of Wildlife Diseases* 30:8–15.
- Aguirre, A. A., T. M. O'Hara, T. R. Spraker, and D. A. Jessup. 2002. Monitoring the health and conservation of marine mammals and sea turtles and their ecosystems. Pages 79–94 in A. A. Aguirre, R. S. Ostfeld, G. M. Tabor, C. House, and M. C. Pearl, editors. *Conservation medicine: ecological health in practice*. Oxford University Press, New York.
- Aguirre, A. A., T. R. Spraker, G. H. Balazs, and B. Zimmerman. 1998. Spirochidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. *Journal of Wildlife Diseases* 34:91–98.
- Balazs, G. H., and S. G. Pooley, editors. 1991. Research plan for marine turtle fibropapilloma. NOAA Technical Memorandum NMFS-SWFSC-156.
- Brooks, D. E., P. E. Ginn, T. R. Miller, L. Bramson, and E. R. Jacobson. 1994. Ocular fibropapillomas of green turtles (*Chelonia mydas*). *Veterinary Pathology* 31:335–339.
- Dailey, M. D., and R. Morris. 1995. Relationship of parasites (Trematoda: Spirochidae) and their eggs to the occurrence of fibropapillomas in the green turtle (*Chelonia mydas*). *Canadian Journal of Fisheries and Aquatic Sciences* 52(Supplement 1):84–89.
- George, R. H. 1997. Health problems and diseases of

- sea turtles. Pages 363–385 in P. L. Lutz and J. A. Musick, editors. The biology of sea turtles. CRC Press, Boca Raton, Florida.
- Herbst, L. H. 1994. Fibropapillomatosis of marine turtles. Annual Reviews of Fish Diseases 4:389–425.
- Jacobson, E. R., J. L. Mansell, J. P. Sundberg, G. V. Kollias, and M. K. O'Banion 1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). Journal of Comparative Pathology 101:39–52.
- Landsberg, J. H., G. H. Balazs, K. A. Steidinger, D. G. Baden, T. M. Work, and D. J. Russell. 1999. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. Journal of Aquatic Animal Health 11:199–210.
- Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd edition. McGraw-Hill, New York.
- Luppa, H. 1977. Histology of the digestive tract. Pages 225–313 in C. Gans, editor. Biology of the reptilia, volume 6. Academic Press, New York.
- Quackenbush, S. L., T. M. Work, G. H. Balazs, R. N. Casey, J. Rovnak, A. Chaves, L. duToit, J. D. Baines, C. R. Parrish, P. R. Bowser, and J. W. Casey. 1998. Three closely related herpesvirus are associated with fibropapillomatosis in marine turtles. Virology 246:392–399.
- Rainey, W. E. 1981. Guide to sea turtle visceral anatomy. NOAA Technical Memorandum NMFS-SEFC-82.
- Seymour, R. S. 1982. Physiological adaptations to aquatic life. Pages 1–51 in C. Gans, editor. Biology of the reptilia, volume 13. Academic Press, New York.
- Smith, G. M., and C. W. Coates. 1938. Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linnaeus). Zoologica (New York) 23:93–598.
- Walker, W. F., Jr. 1959. Closure of the nostrils in the Atlantic loggerhead and other sea turtles. Copeia 1959:257–259.
- White, F. N. 1994. Swallowing dynamics of sea turtles. Pages 89–95 in G. H. Balazs and S. G. Pooley, editors. Research plan to assess marine turtle hooking mortality. NOAA Technical Memorandum NMFS-SWFSC-201.
- Wolke, R. E., and A. George. 1981. Sea turtle necropsy manual. NOAA Technical Memorandum NMFS-SEFC-24.
- Work, T. M., and G. H. Balazs. 1999. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. Journal of Wildlife Diseases 35:804–807.