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PATHOLOGY ASSOCIATED WITH CARDIOVASCULAR TREMATODES AND FIBROPAPILLOMAS IN GREEN TURTLES (*CHELONIA MYDAS*) FROM THE HAWAIIAN ISLANDS

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PREFACE

This report was prepared in part as the result of contracts issued to Dr. A. Alonso Aguirre from 1991-93 by the Southwest Fisheries Science Center Honolulu Laboratory's Marine Turtle Research Program. The report describes gross and histopathologic examination of Hawaiian green turtles, *Chelonia mydas*, with and without fibropapillomas. The cause of fibropapillomatosis, a debilitating and often fatal tumor disease, remains unknown although a viral etiology seems most likely. The disease represents a potentially serious threat to green turtle populations at several locations worldwide, including Hawaii, Florida, and areas of the Caribbean. The present report by Dr. Aguirre supplies comprehensive information on the pathological associations of fibropapillomas and trematode parasites in green turtles resident to the Hawaiian Islands.

Because this report was prepared by independent investigators, its statements, findings, conclusions, and recommendations do not necessarily reflect the views of the National Marine Fisheries Service, NOAA.

George H. Balazs
Zoologist
February 1995

EXECUTIVE SUMMARY

Complete gross and histopathologic examination of 14 turtles collected in the Hawaiian Islands with green turtle fibropapillomas (GTFP) and two control turtles was performed to determine pathologic conditions related to GTFP. All gross and histopathologic lesions were associated with spirorchid trematode infections and GTFP. Turtles were severely emaciated demonstrating a chronic condition. Gross lesions included lobulated tumors of different size classes, serous atrophy of fat, and edema in subcutaneous tissues, pectoral and coracoid muscles. Anasarca, hydropericardium, and pulmonary edema were common findings. A generalized thickening and hardening of major vessels, with partial or complete obliteration of blood flow and thrombi formation were observed in all turtles. Histologically, lymphoplasmacytic endarteritis was observed in most arteries. This lesion was characterized by fibromuscular intimal proliferation, papillary formation, and generalized thrombosis. In several areas the proliferative lesions resulted in total occlusion of the vessels. Multifocal granulomas associated with trematode ova were observed throughout the parenchyma of many organs in all turtles with GTFP. These parasitic granulomas were present in subcutaneous tissue, brain, spinal cord, salt glands, salivary glands, lymph nodes, heart, pericardial sac, lungs, thymus, salivary gland, gastrointestinal tract, mesentery, kidneys, interrenal tissue, spleen, pancreas, urinary bladder, testes, and ovaries. The granulomas were composed of multinucleated giant cells and macrophages engulfing parasitic ova. The neoplastic processes observed in internal organs of some turtles were characteristic of fibropapillomas, fibromas, leiomyomas, myxomas, and fibrosarcomas. Control turtles had no significant clinical signs or gross lesions, and their tissues were within normal limits. It is postulated that when occurring together, spirorchidiasis and GTFP represent a debilitating and fatal syndrome of green turtles in the Hawaiian Islands.

INTRODUCTION

More than 50 species of digenetic trematodes have been reported in green turtles (*Chelonia mydas*) worldwide. From these, 7 genera and 12 species belong to the family Spirorchidae (Digenea: Schistosomatoidea). The life cycle of marine spirorchids is unknown; however, snails and polychaete annelids have been suggested as intermediate hosts shedding cercariae that penetrate the mucous membranes of natural orifices in sea turtles. The adult flukes inhabit the cardiovascular system, primarily the heart, the visceral and mesenteric vessels. Ova are shed through fecal material or urine (Dailey and Morris, 1993; Lauckner, 1985; Smith, 1972). Smith and Coates (1939) first reported the presence of spirorchid trematode ova within green turtle fibropapillomas (GTFP), although the parasites were not implicated as the immediate etiology of the neoplastic condition. Papillomatous hyperplasia was later described associated with *Rhytidodoides similis* flukes in the gallbladder of green turtles (Smith et al., 1941). The ubiquitous presence of trematode ova within the fibrotic portion of the lesions, lead to the hypothesis that GTFP could have a digenetic trematode etiology (Dailey and Balazs, 1987). A survey of trematodes parasitic in Hawaiian green turtles reported four species of intravascular trematodes associated with GTFP (Dailey et al., 1992). These species included *Learedius learedi* (Price, 1934), *Carettacola hawaiiensis* (Dailey et al., 1991), and *Hapalotrema dorsopora* and *H. postorchis* (Dailey et al., 1993).

Clinical signs, gross, and histopathologic lesions for spirorchidiasis in sea turtles have been previously reported (Greiner et al., 1980; Wolke et al., 1982; Rand and Wiles, 1985; Glazebrook et al., 1981, 1989; Norton et al., 1990). Relatively few studies have described an association of spirorchid trematode infections to GTFP (Jacobson et al., 1989; Norton et al., 1990; Harshbarger, 1991; Herbst, 1994). This study reports the pathologic characterization of spirorchidiasis as a debilitating and fatal disease of green turtles when associated with GTFP in the Hawaiian Islands.

MATERIALS AND METHODS

Field Methods

Turtle specimens for this study were obtained from different sources. A total of 10 turtles with extensive fibropapillomas (4 dead and 6 alive) were recovered through the NMFS Hawaiian Sea Turtle Stranding & Salvage Program. In 1992, two freshly dead turtles were found at Palaau, Island of Molokai; one was found ashore at Kaneohe Bay, Island of Oahu; and a fourth one was found ashore at Waialae Beach Park, Island of Oahu. Between June and September 1993, live stranded turtles were recovered at Island of Oahu in the following locations: Kailua Bay (n = 3), Kaneohe Bay (n = 2), and Haleiwa (n = 1). In addition, during October 1992

four green turtles severely affected by GTFP and in poor condition were caught by hand in Kaneohe Bay by snorkelers at Ahu-O-Laka (n = 2) and Mark Reef (n = 2). Two green turtles served as controls including a turtle held in captivity for 5 years at the NMFS Kewalo Research Facility because of traumatic amputation of front flippers, and a pelagic green turtle salvaged as the result of incidental mortality in the foreign driftnet fishery.

All live turtles were transported to the NMFS Honolulu Laboratory for clinical evaluation, euthanasia, and necropsy. Turtles were measured and weighed following techniques previously described (Balazs et al., 1987). Turtles were coded by their degree of tumor severity on a scale of 1-4, 4 being the most severe case. An evaluation of individual tumors was performed based on their size, number and location. The approximate size categories included #1 = detectable patch to 1 cm diameter; #2 = >1 cm to 4 cm; #3 = >4 cm to 10 cm; and #4 = > 10 cm. Location determined degree of tumor severity when vision or ability to feed were impaired (Balazs, 1991).

Gross Pathology and Histopathology

Eleven turtles were euthanized with a lethal intraperitoneal injection of Buthanasia-D Special Solution (Schering-Plough Animal Health Corp., Kenilworth, New Jersey). Thorough necropsies were performed in all 16 green turtles following the protocol described by Wolke and George (1981). After external examination of skin, head, and appendages, the plastron and muscle masses of the pectoral girdle were removed and viscera examined in situ. Standard techniques were followed for the histopathologic evaluation of specimens. Organ sections were fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned 6-μm thick and stained with hematoxylin and eosin. Special stains included Ziehl Neelsen for acid-fast organisms, Macchiavello's for *Rickettsia*, periodic acid Schiff for ground substance of tumors, and Gomori's silver methenamine for fungal hyphae.

Parasitology

Lungs, liver, heart and adjacent vessels, stomach, intestine, bladder, and mesenteric vessels were thoroughly examined for endoparasites in the turtles collected during 1993. Trematodes were placed in a petri dish containing PBS pH 7.4, counted, and identified to species under a dissecting stereomicroscope with external illuminator, following taxonomic keys to spirorchid trematodes (Dailey et al., 1991, 1992, 1993). Cardiovascular spirorchids were air-dried, placed in nalgene cryogenic vials and frozen at -70°C for future study. Other trematodes were transferred through several courses of alcohol-

formalin-acetic acid (AFA) solution, rinsing each one in successively diluted concentrations, and stored in 70% ethyl alcohol. Adult parasites used for identification were stained in Semichon's acetocarmine, dehydrated in a graded ethanol series, and mounted in Hoyer's medium beneath thin coverslips (Dailey et al., 1992).

RESULTS

Green Turtles

Fourteen green turtles demonstrated multiple cutaneous and conjunctival fibropapillomas. A total of 248 external fibropapillomas were counted in 14 green turtles, classified as follows: 60 #1, mean (±SD) 5±6 (range 1-22) tumors/turtle; 146 #2, mean (±SD) 11±8 (range 3-31) tumors/turtle; 34 #3, mean (±SD) 3.5±2.5 (range 1-9) tumors/turtle; and 8 #4, mean (±SD) 1±0.4 (range 1-2) tumors/turtle. The overall degree of tumor severity in males was 3 ± 0.0, and in females averaged 2.8 ± 0.8 (range 1-4). Clinically, the controls were free of GTFP, except for the captive turtle which presented a 2-mm growth in the right nictitating membrane.

Males (n = 4) had a mean (±SD) straight carapace length (SCL) of 68.1±17.3 cm (range 46.0-88.1 cm), and mean (±SD) weight of 41.5 ± 26.7 kg (range 10.0-75.0 kg). Females (n = 10) had a SCL (±SD) of 60.5 ± 10.6 cm (range 50.8-85.7 cm) and mean (±SD) weight of 27.6 ± 19.0 kg (range 15.4-77.2 kg). The captive control was a female with a SCL of 52.3 cm and weight of 16.4 kg. The pelagic control was a 3.5-kg male with a SCL of 28.8 cm.

Parasitology

Three species of cardiovascular flukes (n = 159) of the family Spirorchidae Stunkard 1921 were recovered from the heart and major associated vessels of three turtles collected during 1993. A total of 85 specimens of *Hapalotrema dorsopora*, 67 specimens of *Learedius learedi*, and 7 specimens of *Carettacola hawaiiensis* were identified. Most specimens (68 of *H. dorsopora*, 24 *L. learedi*, and 7 *C. hawaiiensis*) were recovered from a female turtle with GTFP stranded in Haleiwa, Island of Oahu. All specimens of *C. hawaiiensis* were recovered from the hepatic vessels. Multiple infections with two or three parasites were observed in two turtles.

Gross Pathology

Turtles with GTFP had moderate to severe emaciation, cachexia, soft and sunken plastrons, and their carapaces were muddy with algal growth, soft and easy to peel (decalcified).

Extensive serous atrophy of fat with a yellowish, gelatinous appearance was evident. Visceral and pericardial fat was absent or atrophied and subcutaneous tissues were edematous. Pectoral and coracoid muscles were pale, atrophied, and gelatinous. Large amounts (up to 5 liters) of a light yellow-green fluid with a putrid odor were present in the pleuroperitoneal cavity of 5/14 turtles. Hydropericardium and edema of lungs and trachea were observed in 8/14 turtles. Viscera appeared to be anemic and liver in 2 turtles was atrophied. Severe enteritis and hepatitis were observed in one turtle. In 9/14 turtles, generalized thickening and hardening of major vessels including the aortic, pulmonary, mesenteric and hepatic arteries, were observed. In many animals we observed complete obliteration of the lumen of vessels due to endothelial proliferation and thrombi formation. Along the surface of the serosa of small intestine, particularly jejunum and ileum, multiple black spots (1-3 mm diameter) were present in 11/14 turtles with GTFP and in the captive control. These spots were reported as packets of spirorchid ova containing 100-150 eggs (Dailey and Morris, 1993).

Lobulated tumors were present on the skin of the axillary and inguinal regions, neck, head, cloacal area, and between the scales and scutes of turtles with GTFP. Complete and partial loss of vision was observed in 6/14 turtles due to tumors invading the eyes and adjacent tissues. Tumors were also present in the mouth within the palate, sublingual region, glottis, and temporo-mandibular joint of 7/14 turtles. Early growths were pigmented brown to dark grey with a rough surface. Larger tumors had a "cauliflower" appearance or a smooth surface. In 7/14 turtles, tumors were necrotic, hemorrhagic, and heavily infested with piscicolid leeches *Ozobranchus branchiatus* and their eggs. In addition, specimens of the talitroidean amphipod *Hyachelia tortugae* were present in skin and tumors of 3/14 turtles.

Internal tumors and granulomas were evident in 5/14 turtles. Three turtles presented pulmonary tumors of different size classes including 4 X 6 mm, 15 X 23 mm, 20 X 30 mm, 40 X 30 mm, and 120 X 80 mm. These tumors resembled fibromas with yellow-brown, necrotic centers. Smaller tumors were white, fibrotic, firm and solid, and some tumors were filled with a serous fluid. Two turtles had growths and granulomas in kidneys, heart, small intestine, liver, and spleen. A turtle with multisystemic internal and cutaneous fibropapillomas had a necrotic, purulent mass of 100 X 75 mm, extending through the eye into the salt gland and adjacent bone tissues (Fig. 1).

Turtles used as controls were in excellent body condition. Their carapace and plastron were hard, shiny, and with good coloration. Subcutaneous and visceral fat was abundant, about 3-5 cm thick, and dark yellow-grey in color. Pectoral and coracoid muscles were well developed and internal organs in situ had good coloration. No lesions were evident in either control.

Histopathology

Skin

The skin lesions were typical of a fibropapilloma with pseudoepitheliomatous hyperplasia and acanthosis as previously described (Aguirre et al., 1994). Furthermore, numerous whorls of keratinocytes were present within the squamous areas of the hyperplastic epithelium of the fibropapillomas. The neoplasia within the dermal component was characterized by plumb cells with open, round to regular shaped nuclei. Cells were fusiform and surrounded by finely fibrillar cytoplasm. A few heterophils were scattered throughout the tumors. Tumors from 5/14 turtles had a reactive superficial dermis undergoing early neoplastic transformation primarily of a fibroblastic nature, morphologically similar to flat, equine sarcoids (Jubb et al., 1992) (Fig. 2). The tumor of one turtle had stellate cells similar to a myxoma in the deeper areas of the skin, while the more superficial areas were denser with acanthotic epithelium and a moderate degree of spongiosis. Mild to severe ballooning degeneration or microcavitary degeneration with marked intracellular swelling was observed in several tumors. Most skin tumors (240/248) contained granulomata with trematode ova.

Subcutaneous and Adipose Tissues

Proliferative lesions characterized by foci of fibrosis associated with parasitic ova and composed of fibroblastic cells were observed in the subcutaneous tissue of 4/14 turtles. Serous atrophy of adipose tissue was evident in all turtles with GTFP.

Nervous System and Sensory Organs

Brain and Spinal Cord--Multifocal granulomas and lymphocytic infiltration within the vessels and parenchyma of the thalamic region, brain stem, choroid plexus, pineal body, spinal cord, and meninges, surrounded by macrophages and multinucleated giant cells were found in 5/14 turtles. In addition, the meninges had small areas of mineralization.

Eyes--Papillary tumors directly stemming from the limbus and cornea extending to the conjunctiva, histologically similar to ocular fibropapillomas previously described (Brooks et al., 1994), were observed in 6/14 turtles. An extensive amount of pigmentation was present throughout these tumors. Lesions of the corneal epithelium were associated with pressure necrosis. Multifocal, parasitic granulomas were found within these tumors.

Cardiovascular System

Heart--Proliferative multifocal endocarditis characterized by fibrovascular intimal proliferation with papillary formation and lymphoplasmacytic infiltration and thrombosis was observed in

9/14 turtles. Multifocal granulomas with basophilic bodies surrounded by cellular debris, lymphocytosis, and plasma cell infiltration were present within the numerous small crevices of the myocardium. Serous atrophy of pericardial fat and pericarditis associated to spirorchid trematode ova were common findings.

Vessels--A generalized endarteritis and hypertrophy, primarily of carotid, thoracic and abdominal aorta, pulmonary artery, coronary and mesenteric arteries, were present in all turtles with GTFP. These lesions were characterized by an extensive fibromuscular intimal proliferation, papillary formation with vacuolization and mineralization within the outer muscular layer, and subintimal lymphocytic infiltration (Fig. 3). The generalized thrombosis observed was most severe in mesenteric, hepatic, and renal vessels, which contained a moderate amount of lymphoid tissue around the adventitia. In 7/14 cases, the large vessels were obliterated with fibrous connective tissue, causing partial or total occlusion (Fig. 4). Accumulation of mononuclear cells, primarily lymphocytes, plasma cells, and heterophils within foci of proliferation was common. Cartilaginous metaplasia was evident in the adjacent vessels of the heart in 3 turtles. Multifocal, parasitic granulomas were common within the serosa and intima of many vessels.

Respiratory System and Associated Glands

Fibroplasia of alveolar walls (interstitial fibrosis) and severe pulmonary sclerosis, with infiltration of lymphocytes, plasma cells, and heterophils were observed in the lungs of 12/14 turtles. The vessels and interstitial tissue within the walls of the alveolar spaces were characterized by multifocal parasitic granulomas and thrombosis (Fig. 5). In many cases, ova were present within multinucleated giant cells and granulomata surrounding them. Four turtles had lung tumors classified as fibromas and one turtle had a well differentiated fibrosarcoma. The primary cell types of these tumors were the fibroblastic portions of skin fibropapillomas that metastasized to lungs, liver, spleen, and kidneys. Multifocal, parasitic granulomas were observed in the lamina propria of the trachea. Tracheitis with lymphoid infiltration was evident in one turtle. The thyroid gland of 3/14 turtles contained relatively normal and active follicles; however, arteries surrounding the gland had extensive endarteritis and lymphoid cell infiltration, characteristic of other vessels. Thymic lymphoid depletion was observed in 6/14 turtles. Granulomas and endarteritis were also observed in the interstitial tissue of lymph nodes and salt glands of 5/14 turtles.

Gastrointestinal System and Associated Glands

Gastrointestinal Tract--Multifocal parasitic granulomas were found in the esophagus, stomach, and small and large intestines

in all turtles with GTFP. Granulomata were located within the mucosa, lamina propria, submucosa, muscular wall, and serosa (Fig. 6). In addition, most vessels of this region had granulomas with severe endarteritis characterized by fibromuscular intimal proliferation. Numerous fungal hyphae resembling *Candida* were present on the surface of the epithelium of the esophagus in one turtle. A section of stomach in another turtle contained a large pyogranuloma composed of eosinophilic infiltration, parasitic ova, multinucleated giant cells and necrotic, fibroblastic tissue. The mucosa in this area was inflamed and had a polyp on its surface. This polyp was classified as a myxoma. Also, a large, proliferative mass composed of fusiform cells forming interweaving bundles was observed in the jejunum of one turtle. Numerous eggs surrounded by a few macrophages were present throughout the proliferative mass. This mass was recognized as a leiomyoma, originating from the smooth muscle of intestine. The mitotic index of this mass was low. In another turtle, the posterior large intestine contained a proliferative mass identified as a fibrosarcoma. Diffuse granulomata and pyogranulomas were also identified in the cloaca of 6/14 turtles. Multifocal granulomas and endarteritis were also observed in the salivary glands (4/14), tongue (7/14), and pancreas (10/14) of turtles with GTFP.

Liver--Extensive amounts of pigment were observed within the Kupffer cells; parasitic granulomas and necrotic foci in parenchyma with varying degrees of fibroplasia and endarteritis with papillary proliferation in hepatic vessels were observed in 9/14 turtles. Hepatocellular necrosis surrounded by multinucleated giant cells forming a pyogranuloma was evident in one turtle. Adult trematode parasites were identified in the hepatic vessels of 5/14 turtles. A well-differentiated fibroma partially expanding from one of the hepatic vessels within the liver capsule was identified in another turtle.

Spleen--Severe lymphoid depletion with multifocal parasitic granulomas throughout the splenic red pulp was evident in the spleen of 11/14 turtles. Extensive medial hypertrophy and lymphoplasmacytic endarteritis and thrombosis of splenic vessels as described in other tissues were also observed.

Urogenital System and Associated Glands

Multifocal embolic glomerulonephritis was observed in 12/14 turtles. This was characterized by granulomatous lesions, endarteritis, and thrombosis within the renal parenchyma, interstitial tissues, and renal vessels. Parasitic granulomas were surrounded by an extensive accumulation of lymphoid cells. The same lesions were observed in the interrenal tissue (adrenal gland), oviduct, and testes. A mononuclear, multifocal cystitis was observed in five turtles. Lymphocytic plaques in the mucosa of the bladder were associated with parasitic granulomata within the lamina propria, submucosa, and vessels.

Control Turtles

Multiple sections of similar organs examined in the two control turtles had no significant lesions. These sections were in extremely good condition. Parasitic ova and granulomas were absent in all tissues, even though spirorchid trematode ova were confirmed by coproparasitoscopic examination in the captive control.

DISCUSSION

Pathologic lesions caused by spirorchid trematodes and their eggs are well documented for farmed and wild sea turtles in the Atlantic seaboard (Florida to Massachusetts) of the United States (Wolke et al., 1982; Jacobson et al., 1989; Brooks et al., 1994), Australia (Glazebrook and Campbell, 1981, 1990; Glazebrook et al., 1989), Bermuda (Rand and Wiles, 1985), Grand Cayman (Greiner et al., 1980), and Europe (Lauckner, 1985). Proliferative, generalized endarteritis has been associated with chronic irritation caused by adult trematodes and the inhibition of blood flow caused by parasitic granulomas and thrombosis. During this study, a cell-mediated host response was observed in all organs bearing granulomata; characterized by lymphocytic infiltration, macrophages, mononuclear cells, and multinucleated giant cells. This reaction has been observed in farmed and wild sea turtles infected with spirorchids in other parts of the world (Greiner et al., 1980; Rand and Wiles, 1985; Glazebrook et al., 1989; Jacobson et al., 1989; Herbst, 1994). The pathogenesis of this host response was excellently reviewed by Wolke et al. (1982).

The neoplastic processes identified in the internal organs of some turtles were characteristic of fibromas, leiomyomas, myxomas, and fibrosarcomas. A renal myxofibroma in a Florida green turtle (Norton et al., 1990), and fibrotic lung masses (Lucké, 1938) describe internal fibrous nodules in green turtles with GTFP. Harshbarger (1991) also described the presence of fibromas in the lungs, heart, kidney, and gastrointestinal tract in five out of 20 green turtles collected in Florida and Hawaii. The dermal component of these tumors was histologically similar to the cutaneous fibropapillomas. Most fibropapillomas in our study were classified as benign based on morphological appearance; however, architectural and cytological features of malignancy were observed in two cases. Cutaneous tumors were considered malignant (i.e., fibrosarcomas) since they clearly produced metastatic lesions in other organs. In other species, fibroblastic mesodermal cells are induced into pluripotent cells by papillomaviruses (Sundberg and O'Banion, 1989).

The lesions associated with cardiovascular spirorchid trematodes in green turtles have not been described for the Hawaiian Islands, except for an isolated case of a turtle found dead in Kawela Bay, Oahu, in 1985 (Balazs et al., 1987). In

addition, the concurrent association of fibropapillomas and severe trematode infections producing a debilitating and fatal disease syndrome in sea turtles was unknown until recently. Jacobson et al. (1989) concluded that trematode ova observed in one biopsy of a Hawaiian green turtle, were most likely an incidental finding, since no spirorchid eggs were observed in biopsies of six Florida green turtles. During this study, we identified three species of trematodes previously associated with fibropapillomas in Hawaiian green turtles (Dailey and Morris, 1993; Dailey et al., 1991, 1992, 1993). The spirorchid *Neospirorchis schistosomatoides* is also known to cause a pathologic condition in green turtles (Wolke et al., 1982; Rand and Wiles, 1985); however, cardiovascular trematodes from the genus *Learedius* and *Hapalotrema* are most commonly involved in spirorchidiasis (Glazebrook et al., 1989). During this study, the infection of hepatic vessels with *C. hawaiiensis* in one turtle confirmed the observation of Dailey et al. (1991) on the exclusive occurrence of this spirorchid trematode in the hepatic vessels of Hawaiian green turtles.

Although the life cycle of some spirorchid species is known for freshwater turtles (Goodchild and Kirk, 1960; Holliman, 1971), the natural history for the trematodes described herein has not been elucidated. In addition, their effect on the population dynamics of green turtles is unknown. These parasites apparently are widely distributed throughout the benthic habitats of the Hawaiian Islands, where juvenile and adult turtles commonly feed and rest. There is no evidence among the spirorchid parasites of strict phylogenetic host specificity; green turtles apparently act as primary definitive hosts but other sea turtle species are involved in the life cycle (Smith, 1972; Glazebrook et al., 1989).

Recent parasitologic surveys in Hawaiian green turtles afflicted with GTFP (Dailey et al., 1992, 1993) demonstrated prevalences of 80-100% for *H. dorsopora*, 40% for *L. learedi*, and 30% for *C. hawaiiensis*. The distribution of infection with these trematodes apparently was not affected by size, sex, or location of the host; however, the sample was biased towards stranded or moribund animals unable to survive in the wild. Trematode infections easily reach 100% incidence in turtles with GTFP as shown by this and previous studies (Aguirre and Graczyk, 1994). Prevalences of 33% in the Atlantic seaboard (Wolke et al., 1982), 57.1% in Puerto Rico (Fischthal and Acholonu, 1976), and 77.3% in Australia (Glazebrook et al., 1989) have been reported. Concurrent infections of two or more trematodes within one host are common, but it is unknown if this will exacerbate the condition. Our sample size was biased towards Kaneohe Bay. Kaneohe Bay is characterized as an important resting and foraging ground for immature and adult green turtles. Since 1989, over 50% of the turtles captured in this bay manifest GTFP (Balazs and Pooley, 1991). Former studies in this and other areas have shown that turtles approximately 35 cm SCL recruit to benthic habitats

where they spend most of their lives (Balazs, 1980). Previous research has demonstrated that turtles captured in Kaneohe Bay, averaging ≤40 cm SCL were free of fibropapillomas; however, larger turtles averaging ≥45 cm SCL will manifest GTFP (Aguirre et al., 1994, 1994). Furthermore, pelagic turtles are free of fibropapillomas and spirorchid trematodes (Glazebrook et al., 1989). Apparently, turtles become infected with the parasites and the etiologic agent of GTFP when recruiting to nearshore environments (Aguirre et al., 1994). Although many factors influence the rate of parasite infection; trematode infection correlates with recruitment to nearshore environments and a change from carnivorous (macroplankton, fish eggs) to herbivorous diet based on benthic algae and sea grass like *Halophila hawaiiana*. Although sea grass can support large numbers of marine gastropods and most likely, the cercarial stages of the adult trematodes, recent studies on vegetation specimens and snails from the family Neritidae collected in Kaneohe Bay yielded negative results for the presence of cercariae (Dailey, 1991).

The early ballooning degeneration, the intranuclear structures, and the nuclear necrosis of the epithelium in many of the tumors microscopically analyzed, demonstrated lesions highly suggestive of a viral infection. As the lesions progressed, secondary agents were captured within the neoplastic tissue. These agents were dominated by trematode ova resulting in granulomata (Aguirre, 1994). Our research suggests spirorchidiasis is a debilitating and fatal disease of green turtles when associated with GTFP. Further studies will be necessary to identify the primary etiologic agent and determine its relationship to spirorchid trematode burdens and other environmental stressors in Hawaiian green turtles.

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REFERENCES

Aguirre, A. A.
1994. Cellular and hormonal responses to stress and spirorchid trematode eggs of Hawaiian green turtles (*Chelonia mydas*) with and without fibropapillomas. Honolulu Lab., Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Southwest Fish. Sci. Cent. Admin. Rep. H-94-04C, 37 p.

Aguirre, A. A., and T. K. Graczyk.
1994. ELISA test for the detection of anti-blood fluke (*Carettacola, Hapalotrema*, and *Learedius*) antibodies in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas in the Hawaiian Islands. Honolulu Lab., Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Southwest Fish. Sci. Cent. Admin. Rep. H-94-09C, 15 p.

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. J. Wildl. Dis. *30*:8-15.

Aguirre, A. A., G. H. Balazs, B. Zimmerman, and F. D. Galey.
1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian Islands. Mar. Pollut. Bull. *28*:109-114.

Balazs, G. H.
1980. Synopsis of biological data on the green turtle in the Hawaiian Islands. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFC-007, 141 p.

Balazs, G. H.
1991. Fibropapillomas in Hawaiian green turtles. *In*: Balazs, G. H., and S. G. Pooley (eds.). Research plan for marine turtle fibropapilloma. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFSC-156, p. 95-98.

Balazs, G. M., R. G. Forsyth, and A. K. H. Kam.
1987. Preliminary assessment of habitat utilization by Hawaiian green turtles in their resident foraging pastures. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFC-071, 107 p.

Balazs, G. H., and S. G. Pooley (eds.).
1991. Research Plan for Marine Turtle Fibropapilloma. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFSC-156, 113 p.

Brooks, D. E., P. E. Ginn, T. R. Miller, L. Bramson, and E. R. Jacobson.
1994. Ocular fibropapillomas of green turtles (*Chelonia mydas*). Vet. Pathol. *31*:335-339.

Dailey, M. D.
1991. Background presentation on cardiovascular parasitism in Hawaiian green turtles and their possible role as potential etiologic agents of fibropapilloma disease. *In*: Balazs, G. H., and S. G. Pooley (eds.). Research plan for marine turtle fibropapilloma. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFSC-156, p. 83-85.

Dailey, M., and G. H. Balazs.
1987. Digenetic trematodes as possible etiologic agents for fibropapillomas in Hawaiian green turtles (*Chelonia mydas*). Proceedings of the 18th Annual Conference and Workshop of the International Association for Aquatic Animal Medicine, Monterey, California, pp. 46-50.

Dailey, M., and R. Morris.
1993. Relationship of trematode spirorchid parasites and their eggs to the occurrence of fibropapillomas affecting the green turtle (*Chelonia mydas*). Honolulu Lab., Southwest Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Southwest Fish. Sci. Cent. Admin. Rep. H-93-10C, 24 p.

Dailey, M., M. L. Fast, and G. H. Balazs.
1991. *Carettacola hawaiiensis* n. sp. (Trematoda: Spirorchidae) from the green turtle, *Chelonia mydas*, in Hawaii. J. Parasitol. 77:906-909.

Dailey, M., M. L. Fast, and G. H. Balazs.
1992. A survey of the Trematoda (Platyhelminthes: Digenea) parasitic in green turtles, *Chelonia mydas* (L.) from Hawaii. Bull. South. Calif. Acad. Sci. *91*:84-91.

Dailey, M., M. L. Fast, and G. H. Balazs.
1993. *Hapalotrema dorsopora* sp. n. (Trematoda: Spirorchidae) from the heart of the green turtles (*Chelonia mydas*) with a redescription of *Hapalotrema postorchis*. J. Helminthol. Soc. Wash. *60*:5-9.

Fischthal, J. H., and A. D. Acholonu.
1976. Some digenetic trematodes from the Atlantic hawksbill turtle, *Eretmochelys imbricata imbricata* (L.), from Puerto Rico. Proceedings of the Helminthological Society of Washington *43*:174-185.

Glazebrook, J. S., and R. S. F. Campbell.
1990. A survey of the diseases of marine turtles in northern Australia. II. Oceanarium-reared and wild turtles. Dis. Aquat. Org. *9*:97-104.

Glazebrook, J. S., R. S. F. Campbell, and D. Blair.
1981. Pathological changes associated with cardiovascular trematodes (Digenea: Spirorchidae) in a green sea turtle *Chelonia mydas* (L.). J. Comp. Pathol. *91*:361-368.

Glazebrook, J. S., R. S. F. Campbell, and D. Blair.
1989. Studies on cardiovascular fluke (Digenea: Spirorchiidae) infections in sea turtles from the Great Barrier Reef, Queensland, Australia. J. Comp. Pathol. *101*:231-250.

Goodchild, C. G., and D. E. Kirk.
1960. The life history of *Spirorchis elegans*, Stunkard 1923 (Trematoda: Spirorchiidae) from the painted turtle. J. Parasitol. *46*:219-229.

Greiner, E. C., D. J. Forrester, and E. R. Jacobson.
1980. Helminths of mariculture-reared green turtles (*Chelonia mydas*) from Grand Cayman, British West Indies. Proceedings of the Helminthological Society of Washington *47*:142-144.

Harshbarger, J. C.
1991. Sea turtle fibropapilloma cases in the Registry of Tumors in Lower Animals. *In*: Balazs, G. H., and S. G. Pooley (eds.). Research plan for marine turtle fibropapilloma. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFSC-156, p. 63-70.

Herbst, L. H.
1994. Fibropapillomatosis of marine turtles. Ann. Rev. Fish Dis. *4*:389-425.

Holliman, R. B.
1971. Ecological observations on two species of spirorchid trematodes. Am. Mid. Nat. *86*:509-512.

Jacobson, E. R., J. L. Mansell, J. P. Sundberg, L. Hajjar, M. E. Reichmann, L. M. Ehrhart, M. Walsh, and F. Murru.
1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). J. Comp. Pathol. *101*:39-52.

Jubb, K. V. F., P. C. Kennedy, and N. Palmer.
1992. Pathology of Domestic Animals vol. 1, 4th ed. Academic Press Inc., New York, pp. 531-711.

Lauckner, G.
1985. Diseases of Reptilia. *In*: O. Kinne (ed.), Diseases of Marine Animals. Biologische Anstalt Helgoland, Hamburg, Germany, pp. 553-626.

Lucké, B.
1938. Studies on tumors in cold-blooded vertebrates. Tortugas Laboratory Annual Report, Carnegie Institute of Washington 1937/*38*:92-94.

Norton, T. M., E. R. Jacobson, and J. P. Sundberg.
1990. Cutaneous fibropapillomas and renal myxofibroma in a green turtle, *Chelonia mydas*. J. Wildl. Dis. *26*:265-270.

Rand, T. G., and M. Wiles.
1985. Histopathology of infections by *Learedius learedi* Price, 1934 and *Neospirorchis schistosomatoides* Price, 1934 (Digenea: Spirorchiidae) in wild green turtles, *Chelonia mydas* L., from Bermuda. J. Wildl. Dis. *21*:461-463.

Smith, G. M., and C. W. Coates.
1939. The occurrence of trematode ova, *Hapalotrema constrictum* (Leared), in fibro-epithelial tumors of the marine turtle, *Chelonia mydas* (Linnaeus). Zoologica (NY) *24*:379-382.

Smith, G. M., C. W. Coates, and R. F. Nigrelli.
1941. A papillomatous disease of the gallbladder associated with infection by flukes, occurring in the marine turtle, *Chelonia mydas* (Linnaeus). Zoologica, NY *26*:13-16.

Smith, J. W.
1972. The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates and some comparisons with the schistosomes. Helminthological Abstracts, Series A *41*:161-204.

Sundberg, J. P., and M. K. O'Banion.
1989. Animal papillomaviruses associated with malignant tumors. Adv. Viral Oncol. *8*:55-71.

Wolke, R. E., and A. George.
1981. Sea turtle necropsy manual. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SEFC-024, Kingston, Rhode Island, 20 p.

Wolke, R. E., D. R. Brooks, and A. George.
1982. Spirorchidiasis in loggerhead sea turtles (*Caretta caretta*):pathology. J. Wildl. Dis. *18*:175-185.

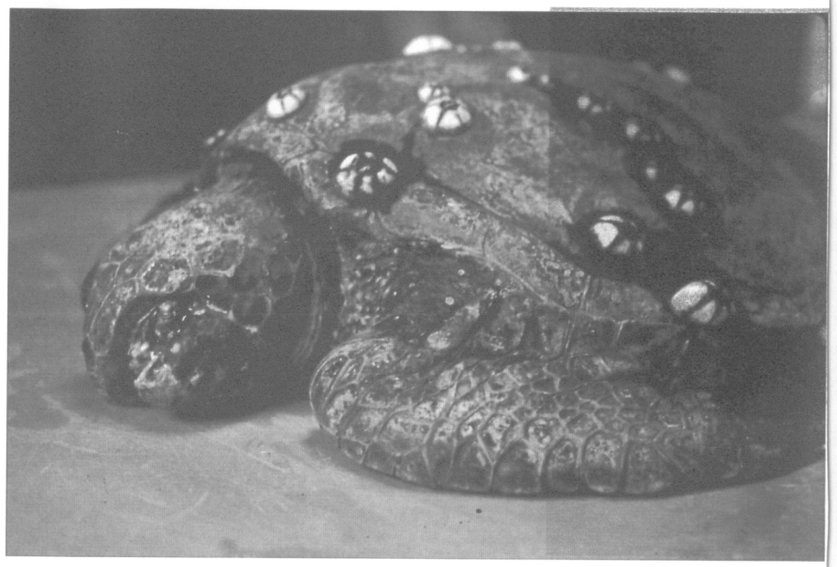

Figure 1.--A green turtle (*Chelonia mydas*) demonstrating severe emaciation and a muddy carapace with algal growth and barnacles *Chelonibia testudinata*. The ocular fibropapilloma, was a necrotic, hemorrhagic mass of 100 X 75 mm, extending through the eye into the salt gland and adjacent bone tissues.

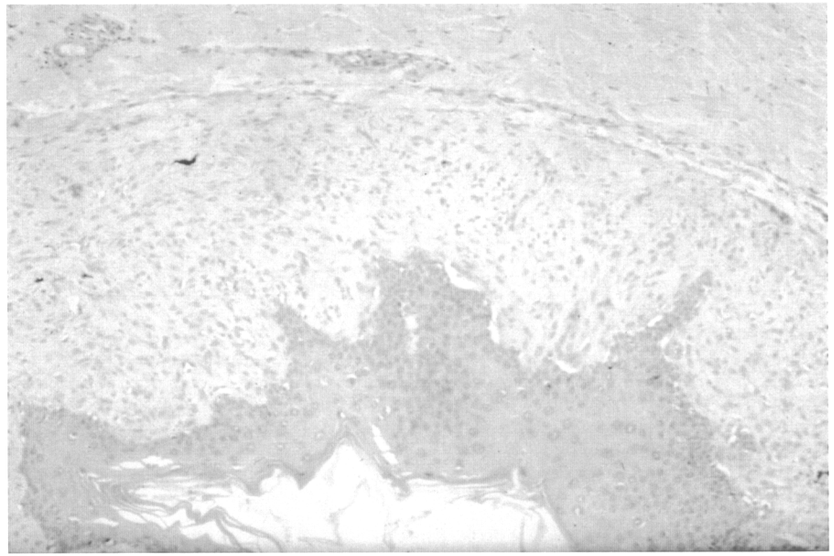

Figure 2.--Fibropapilloma in a green turtle (*Chelonia mydas*) similar to an equine sarcoid. A histologically distinctive lesion, with an epidermis focally acanthotic sending long cords of pseudoepitheliomatous hyperplasia into the proliferating dermal connective tissue. H & E Stain, x 10.

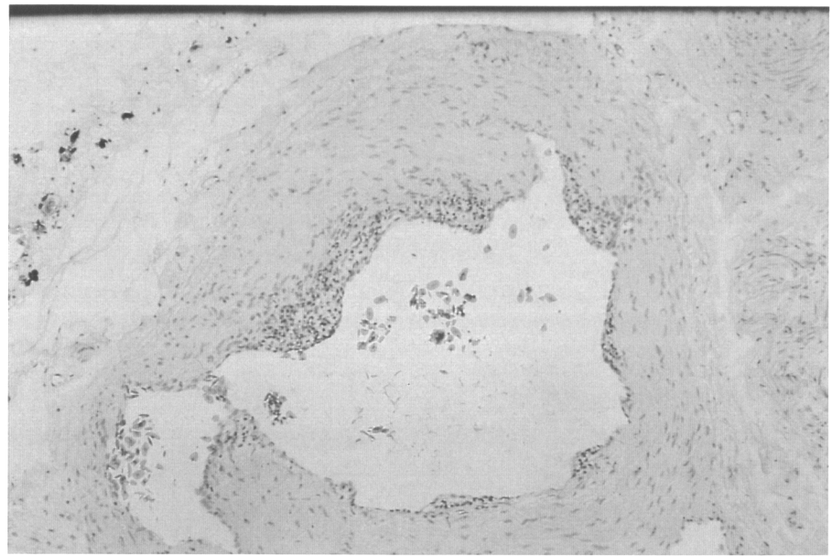

Figure 3.--Proliferative multifocal endarteritis with fibrovascular intimal proliferation, papillary formation, and lymphoplasmacytic infiltration in a mesenteric artery of a green turtle (*Chelonia mydas*). H & E Stain, x 10.

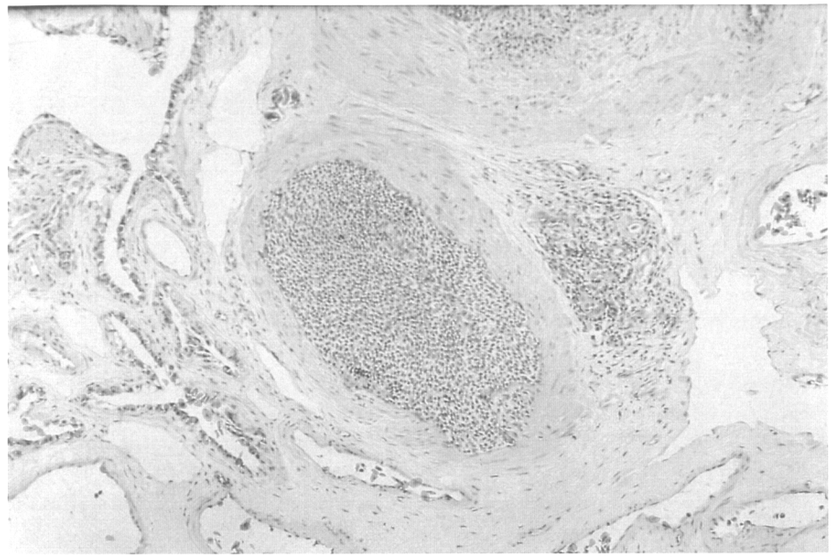

Figure 4.--Pulmonary artery of a green turtle (*Chelonia mydas*) completely obliterated by accumulation of mononuclear cells within foci of proliferation and fibrous connective tissue. H & E Stain, x 10.

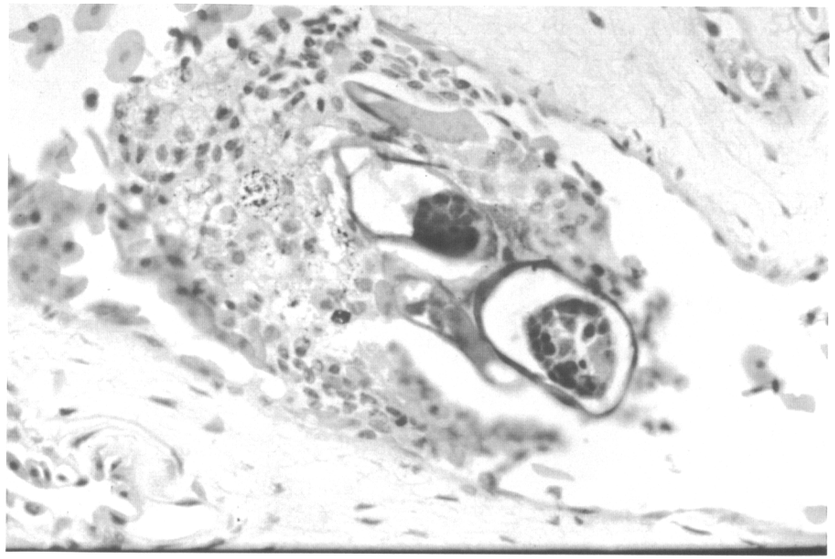

Figure 5.--Spirorchid trematode ova surrounded by a granuloma, an inflammatory response characterized by infiltration of lymphocytes, plasma cells, and heterophils, in a pulmonary vessel of a green turtle (*Chelonia mydas*). H & E Stain, x 40.

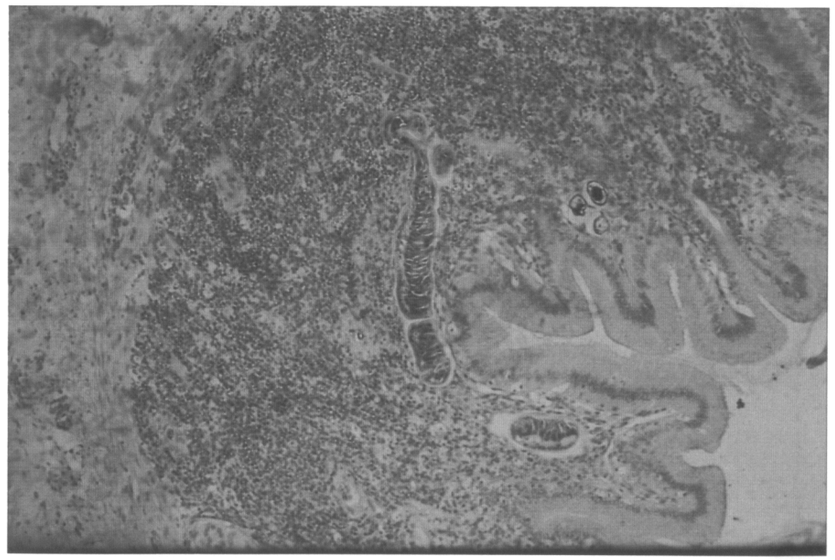

Figure 6.--A vessel of the stomachal mucosa of a green turtle (*Chelonia mydas*) demonstrating an adult spirorchid trematode with ova and moderate cellular infiltration. H & E Stain, x 10.