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Source: Journal of Wildlife Diseases, 34(1) : 91-98

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-34.1.91

Journal of Wildlife Diseases, 34(1), 1998, pp. 91–98

SPIRORCHIDIASIS AND FIBROPAPILLOMATOSIS IN GREEN TURTLES FROM THE HAWAIIAN ISLANDS

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ABSTRACT: Pathologic examination of green turtles (*Chelonia mydas*) from the Hawaiian Islands (USA) was performed to determine the primary cause of mortality. Lesions were associated with fibropapillomatosis (FP) and/or spirorchidiasis (SP) in 16 of 17 green turtles examined. Gross lesions included moderate to severe emaciation, lobulated fibropapillomas of different size classes, serous atrophy of fat, and edema of subcutaneous tissue and muscle. Anasarca, hydropericardium and pulmonary edema were common findings. The neoplastic lesions observed in the gastrointestinal tract, lungs, liver, and kidneys of 29% of turtles examined were histologically characterized as fibromas. A generalized thickening and hardening of major vessels and thrombosis with partial or complete lumen occlusion were observed in turtles with FP and SP. Histologically, lymphoplasmocytic endarteritis was observed in vessels of turtles with both conditions. Multifocal granulomas were associated with trematode ova in the parenchyma of most organs of all turtles with FP and SP. Spirorchidiasis and FP were considered the primary causes of mortality in the turtles examined. Further studies should focus on the pathogenic interaction of both conditions and their synergism as debilitating and fatal diseases in this threatened species.

Key words: *Carettacola hawaiiensis, Chelonia mydas,* fibropapillomas, green turtle, *Hapalotrema dorsopora, Learedius learedi,* pathology, spirorchidiasis.

INTRODUCTION

Fibropapillomatosis (FP) affects sea turtle populations worldwide, sometimes in epizootic proportions (Williams et al., 1994). The etiology of FP remains to be elucidated; however, there is sufficient evidence to implicate one or several infectious agents. A herpesvirus-like virus has been identified and associated with FP in Florida (USA) (Herbst, 1996) and Hawaii (A. Aguirre and T. Spraker, unpubl. data; T. Lipscomb, unpubl. data). More recently, specimens of green turtles (*Chelonia mydas*) in Hawaii were positive for polymerase enhanced reverse transcriptase and negative electron microscopy confirmed the presence of enveloped particles characteristic of a retrovirus (Casey et al., 1996).

The ubiquitous presence of spirorchid trematode ova within the fibrotic portion of the lesions prompted the hypothesis that FP could have a trematode etiology (Dailey and Morris, 1995). Smith and Coates (1939) first reported spirorchid trematode eggs within fibropapillomas, although the parasites were not implicated as the immediate cause of the disease. Papillomatous hyperplasia was later found to be associated with the digenean *Rhytidodoides similis* in the gallbladder of green turtles (Smith et al., 1941). Since that time, more than 50 species of digenetic trematodes have been reported in green turtles worldwide; of these, seven genera and 12 species belong to the family Spirorchidae (Lauckner, 1985). Four species of intravascular spirorchid trematodes, *Learedius learedi, Carettacola hawaiiensis, Hapalotrema dorsopora* and *H. postorchis* are reported in the green turtle (Dailey et al., 1992) from Hawaii. The life cycle of marine spirorchids is unknown; however, snails or polychaete annelids may be intermediate hosts shedding cercariae that penetrate the mucous membranes of natural orifices in sea turtles (Dailey et al., 1992). The adult trematodes inhabit the

cardiovascular system, primarily the heart and the visceral and mesenteric vessels. Eggs are shed through feces or urine (Dailey and Morris, 1995).

Clinical signs and lesions of spirorchidiasis (SP) in sea turtles have been reported previously (Rand and Wiles, 1985); however, the concurrent presence of both FP and SP has not been described. The objective of this study was to summarize the gross and histologic lesions from a group of green turtles affected with concurrent FP and SP in the Hawaiian Islands.

MATERIALS AND METHODS

Wild green turtles for this study were obtained from several sources. Eight turtles with FP were found stranded moribund or freshly dead (within hours) between September 1992 and October 1993 on the Island of Oahu (21°30′N; 157°00′W). In addition, four green turtles severely affected by FP and in poor condition were caught by hand while snorkeling in Kaneohe Bay, Oahu (21°28′N, 157°49′W). One turtle carcass was found tangled in a gill net in Kawailoa, Oahu (21°38′N, 158°05′W); two turtle carcasses were found in a bull pen fishing net at Palau'u (21°06′N, 157°06′W), Molokai. A green turtle held in captivity for 5 yr at the National Marine Fisheries Service (NMFS) Kewalo Basin Research Facility (Honolulu, Hawaii), also was necropsied following euthanasia. This turtle had traumatic amputation of both front flippers. A pelagic green turtle, salvaged as the result of bycatch mortality in a foreign drift net fishery in the North Pacific (30°30′N, 175°02′E) also was necropsied. All turtles were transported to the NMFS Laboratory (Honolulu, Hawaii) for clinical evaluation, euthanasia and necropsy. Turtles were measured and weighed (Balazs et al., 1987). Turtles were categorized into the following size classes: pelagic turtles were under 35 cm straight carapace length (SCL); juveniles were between 35 and 65 cm SCL; subadults were turtles between 65 and 85 cm; and presumed adults were those individuals above 85 cm SCL. All turtles were examined externally for fibropapillomas; if lesions were present, their size, number, and location were noted. Turtles were assigned a fibropapilloma severity score (FPS) on a scale of 0–4, with a FPS = 4 being the most severe case, FPS = 3 being heavily affected, FPS = 2 moderately affected, and FPS = 1 mildly affected. Turtles without FP were given a score of 0. Anatomic site influenced FPS when vision and ability to breathe or feed were considered impaired (Balazs and Pooley, 1991).

Eleven turtles were humanely euthanized with an intraperitoneal injection of pentobarbital with phenytoin (Beuthanasia-D Special, Schering-Plough, Kenilworth, New Jersey) at a dose of 1 ml per 4.5 kg. All 17 green turtles were necropsied following the protocol of Wolke and George (1981). After external examination of skin, head, mouth and appendages, the plastron and muscle masses of the pectoral girdle were removed and viscera examined in situ. Systems (and tissues) collected for detailed histopathologic description included: cardiovascular (pericardium, heart, major vessels); lymphohematopoietic (spleen, thymus, lymphatic nodes, bone marrow); digestive (tongue, esophagus, stomach, intestines, mesenteric vessels and lymph nodes, cloaca); respiratory (trachea, bronchi, lungs); urogenital (kidneys, ureter, urinary bladder, testes, ovary); endocrine (liver, gallbladder, pancreas, interrenal tissue, thyroid and parathyroids, salivary glands, salt glands); and nervous and sensory (brain, spinal cord, meninges, eyes). Sections of all tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and 6 μm sections stained with hematoxylin and eosin (Aguirre et al., 1994). Special stains included Ziehl Neelsen for acid-fast organisms, Macchiavello's for rickettsia, periodic acid Schiff-stain for aldehyde groups, and Gomori's silver methenamine for fungal hyphae (Luna, 1968).

External epizooic associates including marine leeches, amphipods and barnacles were identified (Lauckner, 1985). 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 a phosphate buffered solution pH 7.4, counted, and identified to species using a binocular microscope (Dailey et al., 1992). Other trematodes were fixed in ethanol-formalin-acetic acid (AFA) solution and stored in 70% ethyl alcohol. Adult parasites used for identification were stained in Semichon's acetocarmine and mounted beneath thin coverslips. Voucher specimens were collected and deposited at the Biosystematics and National Parasite Collection (U.S. Department of Agriculture, Beltsville, Maryland, USA; accession number 86340).

RESULTS

Ten female and five male green turtles were collected for full necropsy and histopathologic analysis. Most of these turtles were stranded on Oahu (10 turtles) and

FIGURE 1. Live stranded green turtle (*Chelonia mydas*) from the Hawaiian Islands demonstrating severe ocular and nuchal fibropapillomatosis.

60% of these bordering Kaneohe Bay. Most turtles were juveniles with a mean (±SE; range) SCL of 56 (±2; 46–66)cm and a mean mass of 20 (±2; 10–24)kg. Three subadults and two adults all from Oahu presented a SCL and mass of 69 (±1; 67–71)cm, 39 (±2; 36–44)kg; and 87 (86–88)cm, 76 (75–77)kg, respectively. Most green turtles (94%) presented cutaneous and conjunctival fibropapillomas primarily on the head, neck, axillary region, and front appendages (Fig. 1).

External fibropapillomas (249) measuring 52 (±9; 5–185) mm in diameter were identified distributed by age class as follows: for juveniles 12 (±3; 1–26) tumors; for subadults 28 (±15; 7–58) tumors, and for adults 17 (13–20) tumors. The 52 cm, 17-kg female turtle at Kewalo Basin presented a 2 mm growth in the right nictitating membrane. The pelagic turtle, a male measuring 29 cm SCL and with a mass of 3 kg, had no significant lesions. Small tumors were pigmented brown to dark grey or black with rough and papillary surfaces. Larger tumors had a cauliflower appearance with a fibromatous surface. Many of the growths were necrotic, hemorrhagic and heavily infested with piscicolid leeches *Ozobranchus branchiatus* and their eggs in seven of 15 (47%) turtles. In addition, specimens of the talitroidean amphipod *Hyachelia tortugae* were present in skin and fibropapillomas of three turtles. Complete (5 of 15) or partial (5 of 15) obstruction of vision was observed in 67% turtles with FP and spirorchidiasis due to growths invading the limbus of eyes, conjunctiva and adjacent tissues. Fibropapillomas also were present in the oropharynx and temporo-mandibular joints in eight of 15 (53%) turtles necropsied with apparent destruction of soft and hard tissues.

Grossly, the captive turtle and the pelagic turtle were in excellent body condition. Their carapaces and plastrons were hard, shiny and with normal yellowish-orange coloration. Subcutaneous and visceral fat was abundant, 3 to 5 cm thick, and dark yellow-grey. Pectoral and coracoid muscles were well developed and internal organs appeared normal. Fourteen of 15 turtles with FP exhibited moderate to severe emaciation, cachexia, a soft and sunken plastron and a muddy decalcified carapace with algal growth, indicating that turtles had reduced activity in the wild. Extensive serous atrophy of subcutaneous, visceral and pericardial fat with a yellowish, gelatinous appearance and subcutaneous edema was evident. Pectoral and coracoid muscles were pale, atrophied, and gelatinous. Large amounts (2 to 5 l) of a yellow-green to red fluid were present in the pleuroperitoneal cavity in six of 15 (40%) turtles analyzed. Hydropericardium and edema of lungs and trachea were observed in eight of 15 (53%) turtles. Viscera were pale and appeared to be anemic. Pericarditis, enteritis, hepatitis and cystitis were seen in three turtles.

Generalized thickening and hardening of major vessels (aortic, pulmonary, mesenteric, and hepatic) and total or partial occlusion of the lumen were observed in 10 of 15 (67%) turtles necropsied. The serosa of small intestine, particularly the jejunum and ileum, had multiple black spots (1 to 3 mm diameter) in 12 of 15 (80%) turtles.

Internal tumors were observed in five of 15 (34%) turtles with FP and SP. Pulmo-

TABLE 1. Histopathologic lesions of 15 necropsied green turtles (*Chelonia mydas*) with fibropapillomatosis and spirorchidiasis, from the Hawaiian Islands, 1992–94.

	Histopathologic characteristics						
Body system	Multifocal granulomata	Lymphocytic endarteritis	Thrombosis	Lymphocytosis/ lymphoid depletion	Fibrosis/ sclerosis	Necrosis/ ulceration	Adult trematodes
Cardiovascular	14[a]	14	14	5	7	2	6
Lymphohematopoietic	12	9	9	13	0	0	0
Gastrointestinal	13	11	5	3	2	4	2
Respiratory	13	9	1	2	4	0	1
Urogenital	12	11	1	0	0	0	1
Endocrine	11	10	5	1	0	1	1
Nervous & sensory	6	4	1	1	0	0	0

[a] Number of turtles with lesions.

nary growths of different size classes ranging from 2 × 2 mm to 80 × 120 mm were white and firm. They were solid or filled with a serous fluid or had necrotic centers. Fibromas in stomach, intestine, kidneys, heart, liver, and spleen varying in size from 1 × 2 mm to 50 × 60 mm also were present.

Three species of spirorchid cardiovascular digenetic trematodes (n = 227) were collected from the heart and major vessels of five turtles examined in 1993; these included 125 *Learedius learedi,* 67 *Hapalotrema dorsopora* and 35 *Carettacola hawaiiensis.* Most trematodes (80%) came from three female turtles stranded in Kailua Bay, Oahu, and Kaneohe Bay. All specimens of *C. hawaiiensis* were found in the right auricle and hepatic vessels of one turtle. Multiple infections with *L. learedi* and *H. dorsopora* occurred in two turtles.

Hyperkeratotic orthokeratosis (15 of 15), acanthosis (15 of 15), basal cell degeneration (11 of 15), and spongiosis (3 of 15) were the histopathologic features of skin fibropapillomas. Associated algae (12 of 15), fungi (8 of 15) and bacteria (7 of 15) of unknown pathogenicity were observed on the epidermis and under the keratin layer of most cutaneous fibropapillomas. Tumors in five of 15 turtles (33%) had a reactive superficial dermis undergoing early neoplastic transformation primarily of a fibroblastic nature; these were morphologically similar to flat, equine sarcoids (Jubb et al., 1992). Myxomatous lesions were present in the stratum basale. Mild to severe ballooning degeneration or microcavitary degeneration of the dermal layer with marked intracellular swelling were observed in 11 of 15 (73%) turtles necropsied. Nearly all skin fibropapillomas (240 of 249) contained granulomata with trematode eggs.

Papillary growths directly stemming from the limbus and cornea extending to the conjunctiva were observed in 10 of 15 (67%) turtles necropsied. An extensive amount of pigmentation was present throughout these growths. Lesions of the corneal epithelium were associated with pressure necrosis. All the internal tumors were classified histologically as fibromas.

Histopathologic features of SP are summarized in Table 1. These lesions were generalized in most organs and tissues and consisted of foreign body granulomas, thrombosis and lymphoplasmocytic endarteritis with fibromuscular intimal proliferation (Figs. 2, 3), lymphocyte and plasma cell infiltration and lymphoid cell aplasia. Endocarditis associated with the adult trematodes was diagnosed in 12 of 15 turtles with both conditions present. Pyogranulomas were composed of eosinophils, heterophils, trematode eggs, multinucleated foreign body giant cells, and necrotic fibroblastic tissue (Fig. 4). The black spots

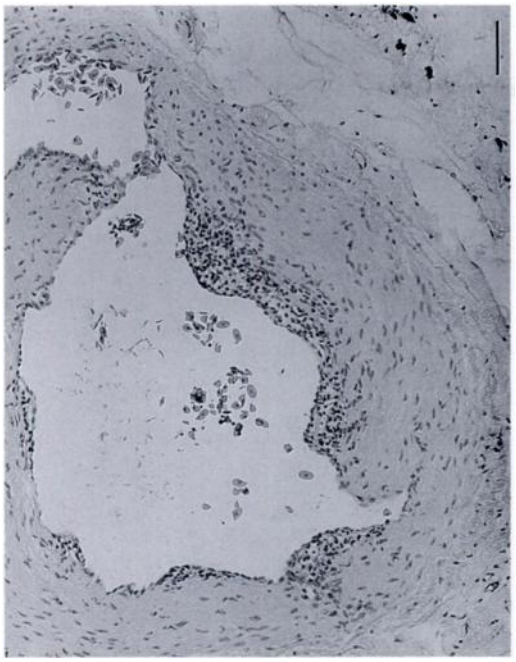

FIGURE 2. Proliferative multifocal endarteritis with fibrovascular intimal proliferation, papillary formation, and cellular infiltration in a mesenteric artery of a green turtle (*Chelonia mydas*) with fibropapillomatosis and spirorchidiasis. H&E. Bar = 100 μm.

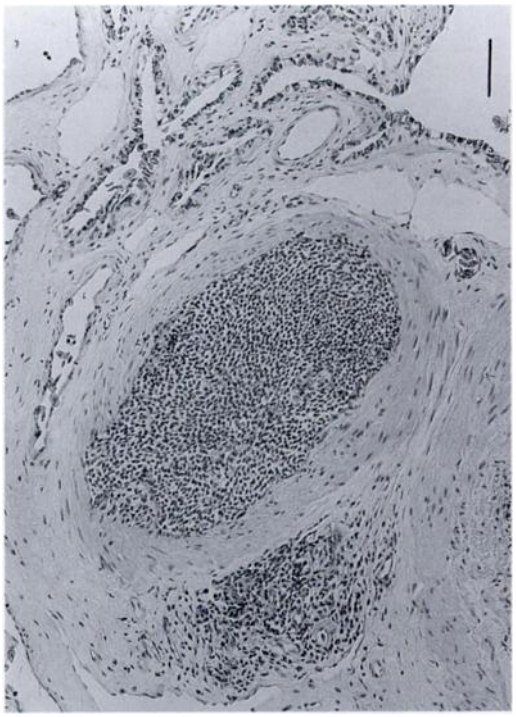

FIGURE 3. Pulmonary artery of a green turtle (*Chelonia mydas*) with fibropapillomatosis completely obliterated by the cellular response and proliferation and fibrous connective tissue caused by spirorchid trematodes. H&E. Bar = 100 μm.

identified in the small intestine of 12 green turtles were identified as packets of spirorchid eggs each containing 100 to 150 eggs. Other incidental lesions identified in turtles with FP and SP included cystitis (27%), pericarditis (7%), rhabdomyolysis (7%), and hepatitis and enteritis (7%).

Internal organs in the captive turtle and pelagic turtle had no significant lesions. Although spirorchid trematode eggs were confirmed by fecal examination in the captive turtle, granulomas were absent in all tissues including the 2 mm palpebral tumor.

DISCUSSION

Skin lesions were characteristic of FP as previously described for green turtles in Hawaii (Aguirre et al., 1994) and Florida (Jacobson et al., 1989). The neoplastic processes observed in the internal organs of several turtles were characteristic of fibromas. A renal myxofibroma in a green turtle in Florida (Norton et al., 1990) and fibrotic lung masses (Schlumberger and Lucke, 1948) have been reported in green turtles with FP. Kaneohe Bay is characterized as an important resting and foraging ground for juvenile, subadult and adult green turtles, the latter of which migrate to breed at nesting in French Frigate Shoals, Northwestern Hawaiian Islands (Balazs et al. 1993). Since 1989, over 50% of the turtles captured in this bay have shown FP (Balazs and Pooley, 1991).

The clinical signs and pathologic lesions of spirorchidiasis are well documented for maricultured and wild sea turtles in the United States and other parts of the world (Wolke et al., 1982; Rand and Wiles, 1985; Glazebrook and Campbell, 1990). The proliferative generalized endarteritis has been associated with chronic irritation caused by the adult trematodes and the inhibition of the blood flow caused by par-

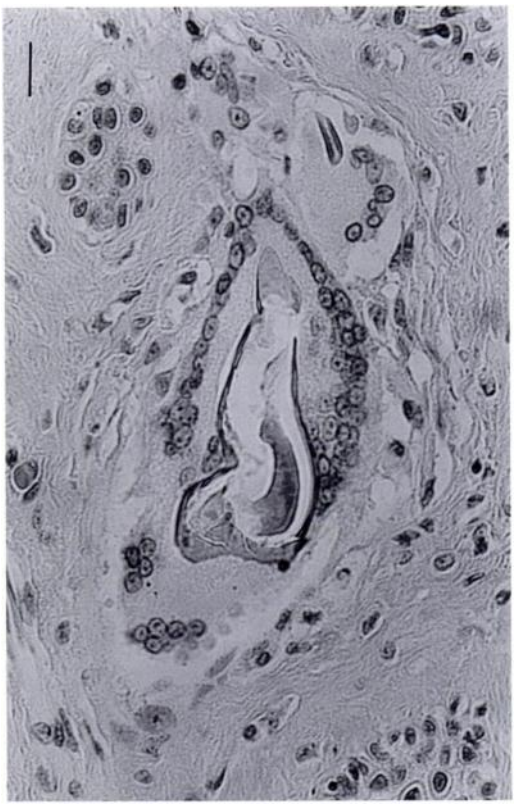

FIGURE 4. Trematode egg surrounded by inflammatory cells in a pharyngeal tumor of a green turtle (*Chelonia mydas*) with fibropapillomatosis and spirorchidiasis. H&E. Bar = 40 μm.

asitic granulomas and thrombosis. During this study, a cell-mediated host response was observed in all organs bearing granulomata; characterized by lymphocytic infiltration, macrophages and multinucleated giant cells. This response resembled those observed in farmed and wild sea turtles infected with spirorchids (Rand and Wiles, 1985; Glazebrook et al. 1989).

With one exception (Balazs et al., 1987), the lesions associated with cardiovascular spirorchid trematodes in green turtles have not been described for the Hawaiian Islands. In addition, the concurrent association of FP and SP as debilitating and fatal conditions in sea turtles was undocumented until recently. We identified three species of adult trematodes previously described in wild green turtles with fibropapillomas in Hawaii (Dailey and Morris, 1995). The spirorchid *Neospirorchis schistosomatoides* also is known to cause a disease in green turtles (Rand and Wiles, 1985); however, cardiovascular trematodes of the genera *Learedius* and *Hapalotrema* are usually involved in SP (Glazebrook et al., 1989).

The impact on the population dynamics of green turtles infected with spirorchids is unknown. These parasites appear to widely infect turtles that reside in coastal benthic habitats in Hawaii for feeding and resting purposes. There is no evidence among spirorchids of strict phylogenetic host specificity; however, green turtles act as primary definitive hosts and other sea turtle species may be involved in the life cycle (Smith, 1972). In the Hawaiian Islands, the green turtle is nearly the exclusive species of sea turtle, although hawksbill turtles (*Eretmochelys imbricata*) occur in small numbers. Among other factors influencing the rate of parasitism, trematode infection has been correlated to recruitment to nearshore environments and change from a carnivorous (macroplankton) to a herbivorous diet based on benthic algae and a sea grass. Recent studies on the sea grass *Halophila hawaiiana* and marine snails from the family Neritidae collected in Kaneohe Bay, yielded negative results for the presence of metacercariae (Dailey and Morris, 1995).

Previous studies in the Hawaiian Islands have demonstrated that turtles approximately 35 cm straight carapace length recruit to coastal habitats where they spend most of their lives (Balazs, 1980). Turtles captured in Kaneohe Bay, averaging ≤40 cm SCL were free of FP and green turtles averaging ≥45 cm SCL had FP. Furthermore, pelagic turtles have been observed free of FP and SP. Apparently, turtles become infected with the parasites and the virus-like agent following movement to nearshore environments (Aguirre et al., 1994; Herbst, 1996).

During the present study, 94% of turtles with fibropapillomas had concurrent trematode infection. A recent study using an

enzyme-linked immuno assay (Graczyk et al., 1995) showed prevalences of 90 to 100% of turtles both with and without FP sampled at Kaneohe Bay. Prevalence of SP in loggerhead turtles (*Caretta caretta*) was reported at 33% in the Atlantic seaboard (Wolke et al., 1982). Prevalence of SP in green turtles free of FP averaged 57% in Puerto Rico (Fischthal and Acholonu, 1976) and 77% in Australia (Glazebrook et al., 1989).

Lesions or embedded trematode eggs in normal tissues were not histologically seen in the captive turtle examined in this study; however, shedding of eggs through feces was demonstrated. Our results suggest that spirorchid trematodes and their eggs are not directly responsible as a primary cause of FP. Following pepsin digestion and egg extraction in tissues of *C. mydas* in Hawaii, Dailey and Morris (1995) found one to 845 eggs/gm of tumor tissue and one to 108 eggs/gm of non-tumor tissue. Trematode eggs may become entrapped within the dermal capillaries of highly vascularized fibropapillomas. Furthermore, immunosuppressed and chronically stressed turtles with clinical FP may be susceptible to secondary SP (Aguirre et al., 1995).

Concurrent cardiovascular trematode infections and FP are recognized as important mortality factors of Hawaiian green turtles considerably reducing the survival of heavily infected individuals. Filterable subcellular agents (i.e., herpesvirus, retrovirus) have been identified as the possible etiology of FP (Casey et al., 1996; Herbst 1996). Our research supports the concept that both diseases are debilitating and fatal to green turtles. However, the interaction of both conditions prove to be highly pathogenic and a primary cause of stranding for Hawaiian green turtles. Further research is necessary to clarify this association. The isolation and characterization of the primary etiologic agent of FP and its interrelationship with SP may provide a better understanding of the ecology and transmission of these and other pathologic stressors affecting marine turtles worldwide.

ACKNOWLEDGMENTS

Logistic and field support provided by D. M. Ellis and S. K. K. Murakawa are deeply appreciated. We also gratefully acknowledge the veterinary clinical assistance of R. Morris. Earlier drafts of this manuscript were reviewed by M. D. Dailey, W. G. Gilmartin and T. M. Work. This research was sponsored in part under contracts No. 40JJNF10175, No. 40ABNF202266 and No. 40ABNF301981 from the NMFS Southwest Fisheries Science Center, Honolulu Laboratory, Hawaii.

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Received for publication 2 September 1996.