Aquatic Mammals 2002, **29.1,** 9–17

Pathological features of the Florida manatee cold stress syndrome

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Abstract

The Florida manatee (*Trichechus manatus latirostris*) is an endangered marine mammal species found primarily in south-eastern coastal waters of the United States. Chronic exposure to cold water produces a cascade of clinical signs and disease processes termed the manatee cold stress syndrome (CSS). No definitive pathological studies have been performed to characterize CSS or define its pathophysiological mechanisms. In this study, pathological features of CSS were characterized in 12 manatees and based on these findings, pathogenic mechanisms were postulated. All age and sex categories were affected by CSS, except the neonatal age category. Emaciation, fat store depletion, serous fat atrophy, lymphoid depletion, epidermal hyperplasia, pustular dermatitis, enterocolitis, and myocardial degeneration were consistent lesions of CSS. These data indicate that CSS is a complex multifactorial disease process that involves compromise to metabolic, nutritional, and immunologic homeostasis and culminates in secondary opportunistic and idiopathic diseases. Treatment for CSS in rescued manatees should address these complex clinical issues. Additionally, these findings are critical for developing future management strategies for this species due to the disappearance or sporadic availability of human-made sources of warm water that manatees habituate to during the winter months.

Key words: manatee, *Trichechus*, endangered species, cold stress syndrome, pathology, mortality, stranding

Introduction

The Florida manatee (*Trichechus manatus latirostris*) is listed as an endangered species in US waters (U.S. Marine Mammal Commission, 2001). Long-term survival of this species is jeopardized by human-related traumatic injuries, perinatal mortality, and degradation/destruction of natural habitat caused by uncontrolled development in Florida (U.S. Fish & Wildlife Service, 2001). Manatees are long-lived and appear to be remarkably resistant to natural disease and the sub-lethal effects of traumatic human-related injury (Buergelt & Bonde, 1983; Buergelt *et al*., 1984; Bossart *et al*., 2002). These traits could partially result from a remarkably efficient and responsive immune system (Bossart, 1999). A notable exception to natural disease resistance is associated with cold weather-associated manatee morbidity and mortality (O'Shea *et al*., 1985; Bossart, 2001). Manatees are tropical to subtropical in distribution and their northern winter range limits are in Florida. They have low metabolic rates, appear to have a limited capacity for heat production and do not tolerate cold water for extended periods of time (Gallivan *et al*., 1983; Irvine, 1983). During the winter, manatees migrate to warm water springs, south Florida canals and lakes, and the warm water effluents of electric power plants and other industrial sources and may rest in these warm water refuges for up to 7 days without feeding (Buergelt *et al*., 1984). Water temperatures below 20°C for extended periods initiate a cascade of clinical signs and chronic disease processes that have been loosely termed the manatee cold stress syndrome (CSS) (Bossart, 2001). Morbidity and mortality are reported to be related to animal size (i.e., age), experience, and migratory abilities (O'Shea, 1988). Thus, adult manatees may handle the effects of cold better than subadults or calves.

Moribund, rescued manatees with CSS are common cases at critical care rehabilitation facilities in Florida. Clinically, manatee CSS involves an often confusing combination of lesions, including

emaciation and infectious disease of the skin and gastrointestinal tract (Bossart, 1999; 2001). Treating rescued manatees with CSS is a common challenge for critical care manatee facilities since the pathogenesis of the syndrome is unknown. Buergelt and colleagues (1984) briefly described the pathologic lesions of two cold-weather associated manatee deaths. However, although CSS is recognized as a cause of manatee mortality, no definitive pathologic studies have been performed to characterize the syndrome or define its possible pathophysiologic mechanisms.

In this report, we present the pathologic findings associated with CSS mortality in 12 manatees that died between November 2000 and April 2001. Based on these findings, we suggest possible mechanisms for the pathogenesis of manatee CSS. Understanding the pathologic features and pathophysiologic mechanisms of the manatee CSS are important for the future management of this endangered species, especially with the disappearance or sporadic availability of human-made warm water sites from powerplants that are habituated by manatees. This information is also needed by clinicians to develop treatment regimens for rescued manatees with CSS in rehabilitation programmes.

Materials and Methods

The winter of 2000–2001 in Florida was particularly severe and associated with persistent cold weather of 0°C (air temperature) lasting one or more weeks. Water temperatures in manatee habitats throughout the central and northern parts of the state remained below 20°C for much of this time period. Between November 2000 and April 2001, 188 manatees were necropsied by the staff of the Marine Mammal Pathobiology Laboratory (MMPL) of the Florida Fish and Wildlife Conservation Commission in St. Petersburg, Florida. Thirty-one of these deaths were categorized as being a result of CSS based on past MMPL criteria involving the correlation with cold weather prior to death and the presence of advanced emaciation, gross skin lesions, and evidence of malnutrition (Buergelt *et al.*, 1984; Bossart, 2001). Twelve of these carcasses with minimum postmortem decomposition were examined for this study.

A complete necropsy was performed at the MMPL on each case following a recommended procedure (Bonde *et al.*, 1983). Morphometrics were determined and age categories estimated by body length that consisted of neonates (1–6 months), calves (6–18 months), subadults (18 months to 5 years), and adults (5+ years) (Ackerman *et al.*, 1995). Tissue sections from the brain, lung, heart, liver, spleen, superficial and deep lymph nodes, thymus (if present), gall bladder, stomach, cardiac gland, pancreas, duodenal diverticula, small intestine, cecum, cecal diverticula, colon, mesenteric fat, kidney, adrenal gland, thyroid gland, skeletal muscle, blubber, and skin were collected for histologic examination. Tissues were placed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin (H&E). Special stains used were Gomori's methenamine silver, Brown and Brenn, and periodic acid-Schiff.

Results

The age and sex distribution of manatees in the study were two calves (1 male [M]/1 female [F]), six subadults (3M/3F), and four adults (1M/3F). Neonates were not represented in the sample population.

Grossly, all of the manatees (n = 12) had evidence of advanced emaciation characterized by prominent head, neck, scapular, rib, and peduncular skeletal features. The ventral abdomen of many animals had multiple longitudinal linear folds and a sunken-in appearance. Additionally, fat depots of the mesentery, omentum, perirenal and periadrenal regions and inner and outer blubber layers of all manatees were markedly depleted (Fig. 1). The remaining fat of the coronary grooves of the heart, perirenal and periadrenal regions, and blubber layers of all animals was translucent and watery with a glistening serosa consistent with serous atrophy of fat.

Nine (75%) manatees (one calf [M], four subadults [2M/2F], four adults) had locally extensive to coalescing or diffuse cutaneous lesions involving primarily the flukes, flippers, and head which extended in a patchy pattern to dorsolateral body regions. The epidermis of the lesions was markedly thickened and consisted of raised, irregular, verruciform, pale grey plaques measuring up to 40 cm in diameter and often contained many fine anastomosing fissures (Fig. 2). Five (42%) of these manatees (three subadults [2M/1F], two adults [1M/1F]) had concurrent locally extensive, raised, firm, brownish-white, cutaneous pustules from 1 to 5 cm in diameter. Some pustules were ulcerated and exuded a yellowish-tan, foul-smelling pus (Fig. 2).

The stomachs and duodenal ampullae of all manatees contained little or no ingesta. The large intestinal lumina of all manatees contained dry, black, and hard faeces or were devoid of faecal matter.

No other significant or consistent gross lesions were observed. Microscopically, significant and

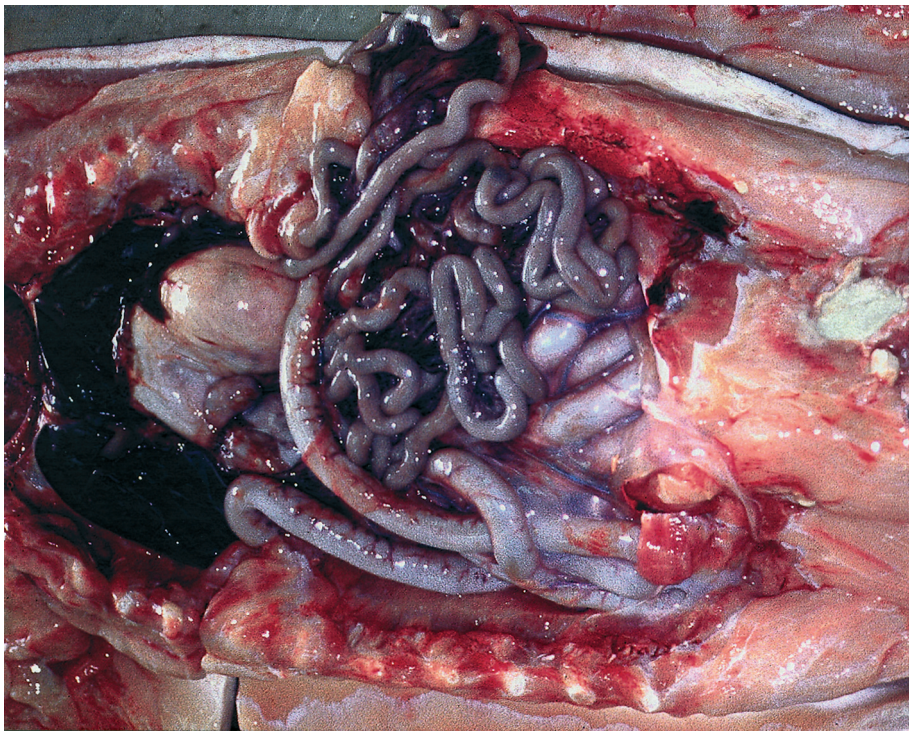

Figure 1. Gross necropsy photo of the abdominal cavity of a CSS manatee. There is marked abdominal fat store depletion with serous atrophy of remaining fat.

consistent lesions were present in the fat, lymphoid tissues, skin, heart and intestinal tract. Serous fat atrophy was present in most remaining fat stores of all manatees. Atrophy was particularly advanced in the coronary grooves of the heart, perirenal fat, and fat surrounding lymph nodes.

Moderate to severe lymphoid depletion was present in all peripheral lymph nodes, spleen and gut- and respiratory-mucosal associated lymphoid tissue (MALT) in 10 manatees (83%) (two calves, four subadults [2M/2F], four adults). The histologic pattern of lymphoid depletion (hypocellularity) involved both follicular and paracortical regions of lymph nodes (Fig. 3); germinal centres and periarteriolar sheath regions of the spleen; and interfollicular areas and germinal centres of MALT. Lymphocytolysis was occasionally present. Additionally, moderate sinus histiocytosis (Fig. 4) or sinus neutrophilia was present in the regional lymph nodes of six manatees (50%) (three subadults [M], three adults [1M/2F]) that had inflammatory changes in either the intestinal tract or lungs.

The skin lesions in the nine manatees grossly described above were characterized by severe, focal to diffuse, epidermal hyperplasia with associated orthokeratotic hyperkeratosis (Fig. 5). A papillated hyperplasic pattern with keratinocyte vacuolar degeneration of the stratum intermedium was present in two subadults (1M/1F) and two adults (F). Additionally, the superficial epidermis was frequently colonized by abundant gram-negative coccobacilli; pleomorphic, agyrophilic, occasionally branching, septate fungal hyphae with parallel walls (Fig. 6); and a few unidentified metazoan parasites.

The cutaneous pustules in all animals had moderate to severe, focal to locally extensive, superficial dermal infiltrates of primarily degenerate and viable neutrophils and occasional macrophages with neutrophilic exocytosis and multifocal keratinocyte necrosis. Pustules occasionally were ulcerated. Intralesional gram-negative coccobacilli or gram-positive cocci were present in suppurative foci of two calves, three subadults [2M/1F] and three adults [1M/2F]. Additionally, inflammation extended into the adjacent subcutaneous tissue in one subadult [M] and one adult [F].

Seven (58%) manatees (one calf [M], three subadults [1M/2F], and three adults [1M/2F]) had moderate, multifocal to diffuse, myocardial degeneration characterized by nuclear 'rowing', cytoplasmic vacuolation, loss of cross-striations, attenuated wavy myofibres and pleomorphic

Figure 2. Gross necropsy photo of the skin of a CSS manatee. The skin has generalized, raised, grey, irregular verruciform plaques of the flipper and ventral abdomen with sloughing. A few focal lesions are ulcerated.

hypertrophic nuclei with prominent nucleoli. Mild, mutifocal, interstitial fibrosis was occasionally present.

Six (50%) manatees (one calf [F], two subadults [1M/1F], three adults [1M/2F]) had a moderate, diffuse, subacute to chronic-active enterocolitis. The lamina propria of the small and large intestines were diffusely infiltrated by neutrophils and macrophages with rare plasma cells. Occasional crypt abscesses and enterocyte necrosis were present and intralesional trematodes were present in one subadult and two adults. Other causal organisms were not seen.

Less frequent, but significant, lesions included moderate to severe, multifocal, chronic-active, suppurative bronchopneumonia in four adults and moderate to severe, diffuse, pancreatic acinar atrophy in one calf [M], two subadults [M], and two adults [F]. The livers of two calves, two subadults (1M/1F) and one adult (M) had scattered pleomorphic, hyaline, intracytoplasmic, hepatocellular inclusions considered consistent with accumulations of keratin intermediate filaments associated with certain types of hepatocellular injury in other species (Cotran *et al*., 1999).

Discussion

All age and sex categories were affected by CSS except the neonatal age category. Similar patterns of CSS mortality were reported recently (Ackerman *et al*., 1995; Rommel *et al*., 2001). In these studies, most CSS mortality involved subadults and adults. Low neonatal mortality was based on the assumption that neonates that survived birth and were accompanied by their mothers had abundant food and an experienced guide to sources of warm water.

Similar consistent pathologic features were represented in all of the remaining age and sex categories. Gross and microscopic evidence consistent with prolonged inanition were present in all cases of CSS. Manatees were markedly emaciated with depleted fat stores and serous fat atrophy. In domestic mammals, these changes are typically associated with the emaciation of inanition and protein-calorie malnutrition (Barker, 1993). The less frequently observed pancreatic acinar atrophy is also common with protein-calorie deficiency in other species (Jubb, 1993). Additionally, evidence of constipation and dehydration were present. These are typical clinical findings in sick manatees

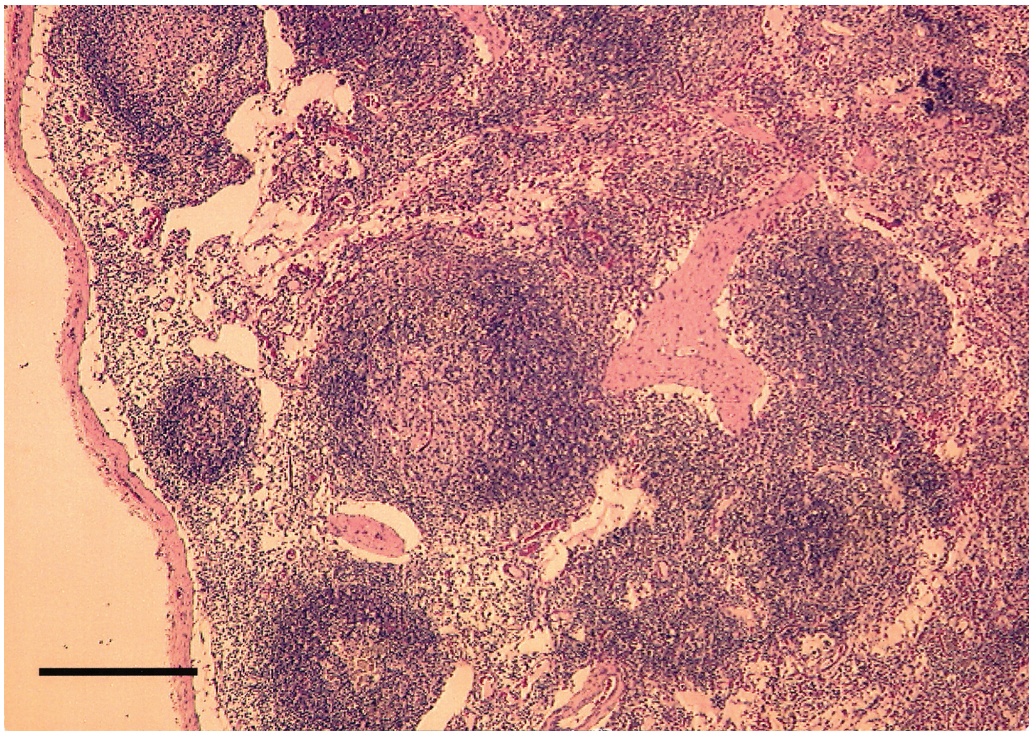

Figure 3. Photomicrograph of a lymph node from a CSS manatee. Notice moderate to severe lymphoid depletion of follicular and paracortical regions. H&E stain; bar=270 microns.

with a decreased food intake (Bossart, 2001). Similar lesions were the only changes mentioned in two cases of CSS from the only previous published study (Buergelt, 1984). In the present study, however, additional consistent and noteworthy lesions were observed in manatees with CSS.

Eighty-three percent of the manatees with CSS had moderate to severe lymphoid depletion of lymph nodes, the spleen and MALT. Lymphoid depletion results from increased destruction of lymphocytes (e.g., sepsis, infectious agents, drugs, and excessive and prolonged endogenous glucocorticoid release associated with stress,) and/or decreased lymphocyte production (e.g., nutritional deficiencies and congenital disease) (Searcy, 1988; Aster & Kumar, 1999). The histological pattern of lymphoid depletion in CSS manatees involved lymph node follicular and paracortical regions, splenic germinal centre and periarteriolar sheath regions and MALT interfollicular and germinal centre regions. These histological sites represent both B and T cell predominant areas in other mammals (Tizard, 1992). In domestic animals and other wildlife species, a similar histological pattern of lymphoid depletion has been reported with combined B and T cell immune deficiencies (Bossart, 1984; Leighton, 1986; King, 1986; Searcy, 1988; Valli, 1993). Thus, in CSS manatees, the pattern of depletion suggests compromise to both the humoral and cell mediated immune systems. Systemically, multiorgan inflammatory disease in CSS manatees (e.g., generalized infectious dermatitis, enterocolitis, and infectious bronchopneumonia) was further evidence that suggests combined deficient immune function. Particularly, the superficial colonization of the epidermis in 50% of the manatees by a striking variety of bacterial, fungal, and metazoan parasites indicated dysfunctional dermatologic primary immune surveillance. Interestingly, enterocolitis in non-CSS manatee calves is a common clinical problem also associated with apparent immunologic deficiencies (Bossart, 2001).

Experimental evidence exists for cold-water stress related immune suppression in laboratory mammals. Chronic cold-water stress in mice causes a reduction in thymic and splenic lymphocytes, decreased T cell blastogenesis, reduced natural killer cell activity, peritoneal macrophage dysregulation, and decreased B cell responsiveness following antigenic stimulation (Cheng *et al.*, 1990). Rats exposed to chronic cold stress have increased levels of ACTH and corticosterone and a pronounced suppression of the humoral immune response (Groundasheva *et al.*, 1994). Lymphocytolysis,

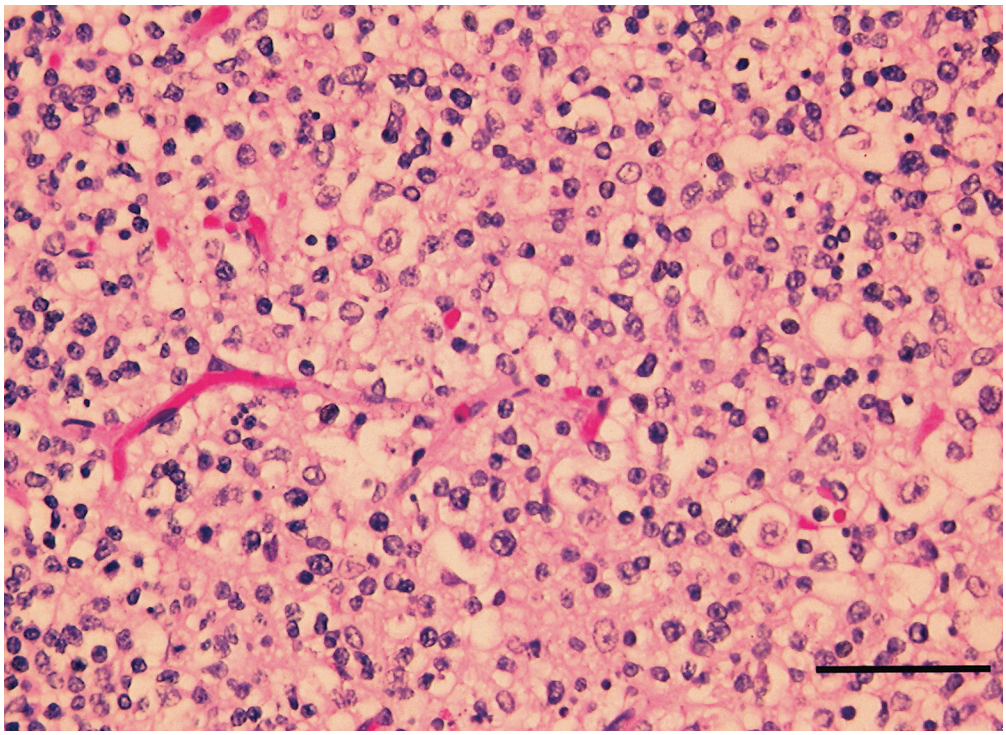

Figure 4. Photomicrograph of a lymph node from a CSS manatee. Marked lymphocyte hypocellularity and medullary sinus histiocytosis are present. H&E stain; bar=100 microns.

which was observed in CSS manatees, also can be a result of excessive glucocortocoid release (Hillhouse *et al*., 1991). Therefore, in manatees, prolonged exposure to cold could cause widespread lymphoid depletion and immune suppression due, at least in part, to excessive and prolonged stress-related glucocorticoid release and compounded by nutritional deficiencies associated with prolonged inanition. Further clinical studies of CSS are required to confirm this speculation.

Additionally, in a preliminary clinical immunologic study, immune suppression was described in CSS manatees (Walsh *et al*., 2002). Lymphocytes isolated from CSS manatee blood revealed significant functional impairment. Lymphocyte proliferation stimulation indices from these animals were significantly lower than the control responses from healthy free-ranging manatees. This study also demonstrated that *in vitro* exposure of CSS manatee lymphocytes to red tide toxins reduced lymphocyte proliferation even further, suggesting that multiple stressors (i.e., combined cold temperatures and red tide toxin exposure) have even greater effects on immune function in these animals. Since red tide blooms occur in winter months, the combination of CSS and exposure to red tide toxins could have a synergistic effect on immune suppression (Bossart *et al*., 1998).

The widely distributed epidermal hyperplasia was another consistent lesion associated with CSS. The cause of this unusual lesion could not be determined. Increasing epidermal thickness could represent an adaptive response to insulate against cold temperatures. Protein-calorie deficiency, fatty acid deficiency, some hypovitaminoses and endocrinopathies including hyperadrenocorticoidism can cause similar epidermal changes in domestic animals (Yager & Scott, 1993). Together with increased ACTH and corticosterone, increased thyroxin levels have been reported in laboratory mammals exposed to cold (Groundaseva *et al*., 1994). The latter is an attempt to increase body temperature via metabolic pathways. Such nutritional and/or endocrine changes could provide a pathophysiological mechanism(s) for the CSS epidermal lesions. Thus, future studies to evaluate these parameters in recovering CSS manatees could provide clues to the cause of the dermatological and lymphoid lesions.

The skin lesions also have gross and histological features consistent with forms of cutaneous papillomavirus (PV) infection seen in humans and

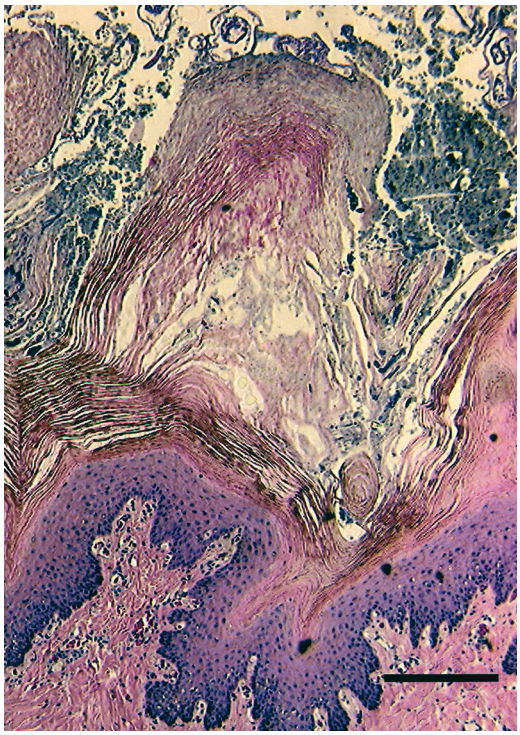

Figure 5. Photomicrograph of skin from Fig 2. There is severe orthokeratotic hyperkeratosis and epidermal hyperplasia with a mild multifocal superficial dermal mononuclear inflammatory cell infiltrate. H&E stain; bar=280 microns.

domestic cats with varying degrees of immunosuppression, particularly humans with epidermodysplasia verruciformis and iatrogenic transplantation immunosuppression (Sundberg *et al*., 2000; Bossart *et al*., 2002). We recently described a novel disseminated cutaneous papillomatosis caused by PV infection in a group of manatees that had evidence of cell-mediated immunosuppression postulated to be secondary to long-term exposure to cool water temperatures between 22.5–23.5°C (Bossart *et al*., 2002). This infection may be latent with recrudescence occurring after immunosuppression secondary to prolonged exposure to cool water. The papillomas in these manatees extended from the basal layer of epidermal keratinocytes to the outermost stratum corneum. Concern exists that under conditions of disseminated papillomatosis activated by immunosuppression, the structural and functional integrity of this abnormal epithelium is compromised as a protective barrier against the environment and that this could result in opportunistic infections. Presently, we are conducting investigations to determine if the manatee PV is associated with the skin lesions of CSS.

The cause and functional significance of the myocardial lesions is unknown. No pathological evidence of myocardial decompensation was present in any CSS case. In other marine mammal studies, similar, but more severe, lesions consistent with idiopathic dilated cardiomyopathy with evidence of congestive heart failure have been described (Bossart *et al*., 1985; Meisner, 2002). In these cases, metabolic (e.g., thyroid hormone imbalance or diabetes mellitus) and/or nutritional (deficiencies in taurine, L-carnitine, thiamine) aetiologies were speculated to be causal factors. Hence, the myocardial lesions associated with CSS could represent a subclinical, but progressive disease related to metabolic and/or nutritional factors associated with the observed advanced emaciation.

Our pathological findings indicated that manatee CSS is a complex multifactorial disease process that involves the integration of metabolic, nutritional, and immunologic factors, often with secondary opportunistic infectious disease and lesions of present unknown etiology. Based on these findings, the pathogenesis of manatee CSS appears to involve a cascade of pathophysiological components that initially involves chronic exposure to water temperatures below 20°C. This exposure, in turn, likely results in lethargy, decreased food intake, dehydration, and constipation which further compromise nutritional, metabolic, and immunologic homeostasis. The latter would ultimately result in immunologic compromise predisposing CSS manatees to opportunistic infections and other unusual lesions that could have infectious, nutritional, and/or metabolic components. Therefore, treatment for rescued manatees with CSS should address these specific factors with special emphasis on re-establishing normal gastrointestinal function and fluid/nutritional status.

The long-term sublethal effects of CSS could be more insidious, involving increased susceptibility to disease and impacts on fecundity and calf survival. The preliminary evidence of the combined suppressive immunologic effects of CSS and exposure to red tide toxins needs further investigation as exposure to cold and red tide toxins have become important recent causes of manatee mortality in Florida (Bossart *et al*., 1998; Walsh *et al*., 2002).

Understanding the pathophysiologic mechanisms of manatee CSS is becoming critically important for developing future management strategies for this endangered species. The availability of a network of warm water refuges could well be one of the most important and challenging long-term needs for manatees (U.S. Marine Mammal Commission, 2001). Most manatees rely on natural or artificial warm water refuges to survive cold winters and typically return to the same refuges year after year. Over the past 30 years in Florida, warm water

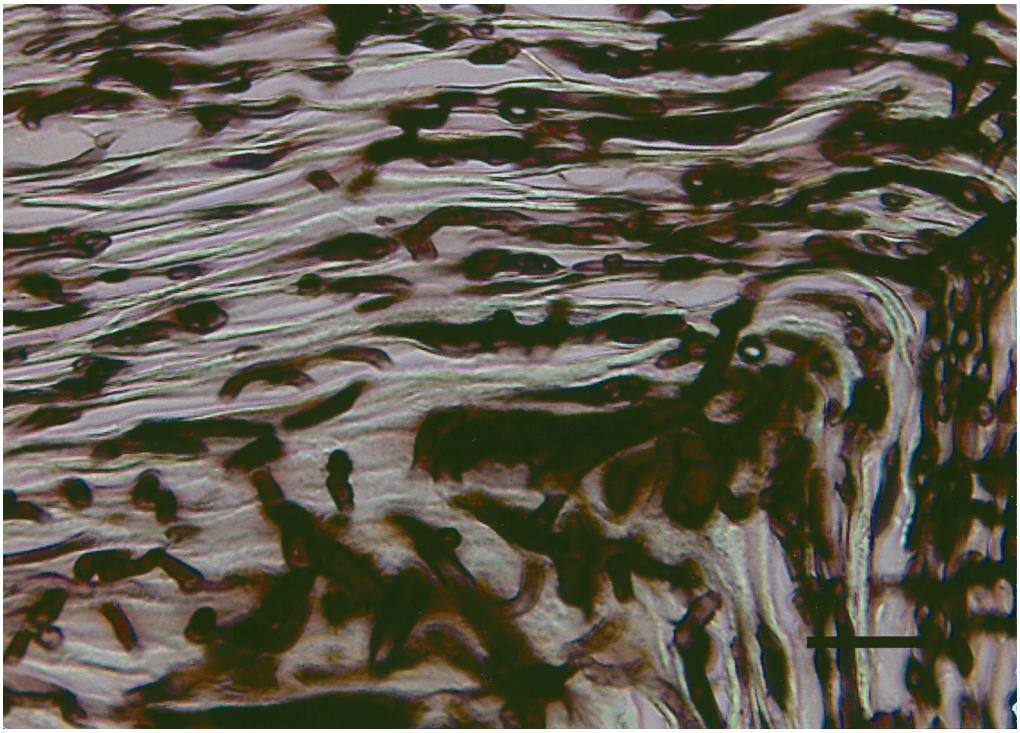

Figure 6. Higher power photomicrograph of Fig 5. Myriads of septate branching fungal hyphae with parallel walls are present within the hyperkeratotic layers. Gomori's methenamine silver stain; bar = 50 microns.

effluent sources at electric power plants have provided life-sustaining refuges for manatees during the winter. This is particularly significant because winter manatee counts at single power plant warm water refuges may exceed 500 animals or approximately 15% of the remaining population (U.S. Marine Mammal Commission, 2001). Serious concerns exist about the inevitable shutdown and/or deregulation of aging power plants that manatees have become dependent on in cold weather (U.S. Fish and Wildlife Service, 2001). Because manatees are unable to adapt quickly to changes in the availability of warm-water, the elimination of these warm water refuges could have profound effects on increasing mortality and population distribution, thus impacting an already compromised species.

Acknowledgments

The authors acknowledge the assistance of the staff of the Marine Mammal Pathobiology Laboratory, Florida Fish and Wildlife Conservation Commission, St. Petersburg, Florida for their invaluable help with the gross necropsies from this study.

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