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# PATHOLOGIC AND PARASITOLOGIC FINDINGS OF COLD-STUNNED KEMP'S RIDLEY SEA TURTLES (*LEPIDOCHELYS KEMPII*) STRANDED ON CAPE COD, MASSACHUSETTS, 2001–2006

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**ABSTRACT:** Necropsy reports for 28 stranded, cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*) that died between 2001 and 2006 were reviewed retrospectively. Gross and microscopic lesions were compiled to describe the pathologic and parasitologic findings in turtles that were found freshly dead on the beach or that died within 48 hr of stranding. Anatomic lesions of varying severity were identified in each of the examined turtles and were identified in tissues of the alimentary, respiratory, integumentary, nervous and sensory, and urogenital systems in order of decreasing frequency. Necrotizing enterocolitis and bacterial or fungal pneumonia were the most frequently encountered lesions that were considered clinically significant. Parasites and parasitic lesions were identified primarily in tissues of the alimentary system and included intestinal cestodiasis and parasitic granulomas containing larval cestodes or nematodes. Postlarval cestodes were also found in the coelom of two turtles. In many cases, the extent and severity of lesions were judged to be insufficient to have solely caused mortality, suggesting that additional factors such as metabolic, respiratory, and electrolyte derangements; hypothermia; and drowning may be important proximate causes of death in cold-stunned turtles. Results of this study provide insight into pathologic conditions that may be of clinical relevance to rehabilitation efforts for cold-stunned sea turtles.

**Key words:** Cold-stunned, Kemp's ridley, *Lepidochelys kempii*, pathology, parasitology, sea turtle, stranding.

## INTRODUCTION

The Kemp's ridley sea turtle (*Lepidochelys kempii*) is the smallest and rarest species of sea turtle, with only 2,000–3,000 adult females remaining (Márquez-M. et al., 2005). Although adults are generally found along the coast of the southeastern United States and Gulf of Mexico, juveniles may migrate to the northeastern coast of the United States during summer (Morreale and Standora, 2005). Juveniles that do not leave northern waters in autumn are susceptible to severe hypothermia as water temperatures rapidly drop, a condition referred to as "cold-stunning" (Gerle et al., 2000). Cold-stunned Kemp's ridley turtles are often found stranded along beaches of Cape Cod, Massachusetts, USA, from Novem-

ber to late December (Still et al., 2002; Wyneken et al., 2006). The reasons for these cold-stunning events are not completely understood; however, it is thought that geographic, oceanographic, and meteorologic conditions are involved (Still et al., 2005). Unfortunately, 35% to 85% of cold-stunned turtles are dead when they are found on the beach (Gerle et al., 2000; Turnbull et al., 2000).

Several reports have generally described pathologic conditions seen in cold-stunned Kemp's ridley turtles (Matassa et al., 1994; Sadove et al., 1998; Smith et al., 2000; Turnbull et al., 2000; Wyneken et al., 2006). Other more detailed reports have described pathologic conditions found in individual cold-stunned Kemp's ridley turtles during rehabilitation (Harms et al., 2002; Manire

et al., 2002). However, detailed pathologic and parasitologic findings from a series of cases have not been reported. This study was conducted to document pathologic and parasitologic conditions present at the time of stranding and in the immediate poststranding period for a series of cold-stunned Kemp's ridley turtles. The results provide insight into the causes of death for cold-stunned turtles, as well as clinically relevant information for immediate poststranding rehabilitation efforts.

## MATERIALS AND METHODS

### Sample collection

Medical records, necropsy reports, and histopathologic descriptions of Kemp's ridley turtles that stranded between 2001 and 2006 were reviewed retrospectively. Turtles were recovered from Cape Cod, Massachusetts, beaches (42°N, -70°W) by staff and volunteers of the Massachusetts Audubon Society. When found on the beach, each turtle was weighed and measured, and core body temperature was recorded. Turtles that were found alive were treated using established protocols for the medical care of cold-stunned sea turtles (Wyneken et al., 2006). Only turtles that were found dead on the beach or that died within the first 2 days of hospitalization were included in the study. Dead turtles were refrigerated at 8 C, and necropsies were performed within 24 hr.

### Histopathology

Tissue samples were collected at necropsy from coelomic viscera, brain, eye, shell, and skin, including all grossly identified lesions, and then fixed by immersion in 10% neutral buffered formalin. Formalin-fixed tissue samples were submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (University of Connecticut, Storrs, Connecticut, USA) and were grossly evaluated, trimmed to fit plastic cassettes, and then processed routinely for paraffin embedment. Tissue sections were cut at 4  $\mu$ m, mounted on glass slides, and stained with hematoxylin and eosin or hematoxylin, phloxine, and saffron, implementing a modified version of the phloxine-saffron counterstain, which uses a nonaqueous saffron dye to progressively displace phloxine dye to stain collagenous tissues (Sheehan and Hrapchak, 1980). Histologic sections were examined by brightfield micro-

scopy, and all histopathologic lesions subsequently identified were described and reported regardless of perceived significance to the stranding event or clinical history.

### Parasitology

Gross examination for metazoan parasites was conducted under a dissecting microscope with tissues submerged in 0.9% saline. Cysts discovered on the serosal surface of organs were dissected using fine-tipped forceps and a hypodermic needle until the blastocyst was free of the host capsule, and then further manipulated until the plerocercus was freed from its blastocyst. The plerocercus was observed until its tentacles were extended, at which time it was immediately pipetted into warm 70% ethanol for fixation or examined microscopically. Tetrahyllidean plerocercoids were collected on glass coverslip mucosal scrapings and examined alive with brightfield microscopy. Nematodes and postlarval cestodes were washed in saline, examined, and then heat fixed in boiling 0.9% saline for 5 sec and transferred to 70% ethanol for storage. Nomenclature and morphologic terminology were based on Palm (2004). Epibionts were identified based on criteria described by Zullo (1979).

Metazoan parasites observed in histologic section were assigned to one of several major phyla of metazoan tissue parasites (e.g., Platyhelminthes and Nematoda) based on characteristic histologic features as described by Chitwood and Lichtenfels (1972) and complemented by Gardiner and Poynton (1999). Platyhelminth parasites were further characterized as members of Trematoda or Cestoda in instances where differentiating anatomic features were identifiable in histologic section.

## RESULTS

Twenty-eight turtles met the inclusion criteria for the study. Turtles were found stranded between 3 November and 22 December of the respective years of their strandings. Turtle body temperature at the time of stranding ranged from 3 C to 12 C (mean=7 C, SD=3 C). All turtles were juveniles, with body masses ranging from 1.67 kg to 3.6 kg (mean=2.65 kg, SD=0.51 kg). Straight carapace length (SCL) ranged from 22.2 cm to 30.7 cm (mean=25.4 cm, SD=2.13 cm). Eleven turtles were found freshly dead; 10 turtles died

on the first day of hospitalization; and seven turtles died on the second day of hospitalization. Thirteen turtles were confirmed to be female, and eight turtles were confirmed to be male by histologic examination of gonads. No information on gender was recorded for seven turtles.

Epibiota included unidentified algae on the head and carapace (eight specimens), barnacles (*Balanus venustus* and *Platylepas hexastylus*) on the plastron (one to three barnacles per turtle, four specimens), and common slipper shell (*Crepidula fornicate*; one specimen; Zullo, 1979).

Seven turtles had previously healed injuries. Of these, five had partial amputation of a single limb, and two had triangular defects along the caudal margins of the carapace. Enophthalmos was present in five turtles, and unilateral periocular hemorrhage was present in two. Nine turtles had moderate, bright red to purple discoloration of the ventral skin of the limbs, and 10 turtles had moderate, red to purple discoloration of the plastron. Two turtles had sand in the oral cavity, eyes, or cloaca. Mild to moderate cloacal eversion with hyperemia of the cloacal mucosa was present in two turtles, and one had blood in its oral cavity.

Five turtles were subjectively thin with markedly reduced coelomic fat. The gastrointestinal tract contained no ingesta in 12 turtles, and only yellow to orange mucus in four turtles. Ingesta in the other specimens included sand (four specimens), unidentified bivalves (four specimens), stones (two specimens), blue mussels (*Mytilus edulis*, two specimens), unidentified crab parts, spider crab (*Libinia emargina*), unidentified plant material, knotted wrack (*Ascophyllum* sp.), Acadian hermit crab (*Pagurus acadianus*), horseshoe crab (*Limulus polyphemus*), rockweed (*Fucus vesiculosus*), common slipper shell, sand dollar (*Echinarachnius parma*), jingle shell (*Anomia simplex*), soft-shelled clam (*Mya arenaria*), green crab (*Carcinus maenas*), and unidentified jellyfish (one specimen each; Andrew and Fredland, 2001). In five turtles, mod-

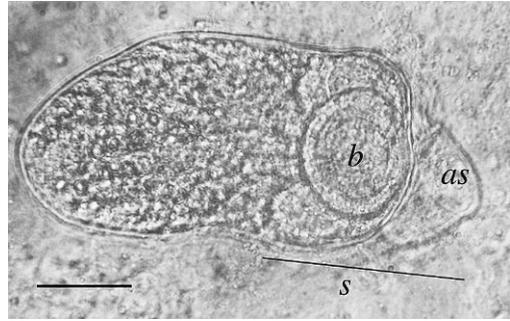


FIGURE 1. Tetraphyllidean plerocercoid (presumptive) from the small intestinal mucus of a Kemp's ridley sea turtle. s = scolex; b = bothridium; as = apical sucker. Bar=0.1 mm.

erate numbers of tetraphyllidean cestode plerocercoids were identified microscopically in the surface mucus of the small intestine. Identification was based on size, clear vacuolated body, and four sessile, undivided, anterior, round to oval bothridia with a single apical sucker (Fig. 1). Both everted and inverted forms were observed. In two cases, the plerocercoids were associated with a matrix of plant material, algae, and bacteria, forming from 0.3 to 0.6-cm-diameter green, mucoid globules free in the intestinal lumen.

All turtles had moderate numbers of white, yellow, or tan, 2–4-mm diameter parasitic cysts on the serosal surfaces of various organs, including small intestine (11 specimens), stomach (10 specimens), liver (four specimens), coelomic peritoneum (four specimens), colon (three specimens), esophagus (two specimens), epicardium (two specimens), trachea (one specimen), and urinary bladder (one specimen; Fig. 2). These cysts contained trypanorhynchian cestode plerocerci (Fig. 3). Yellow or tan cysts contained dead organisms, and cysts were not associated with gross pathologic changes. Live plerocerci were motile and had two broad, oval bothria. The scolex was small (0.46 mm in length  $\times$  0.30 mm in maximum width at the pars bothrialis), elongate, and the pars vaginalis was longer than the pars bothrialis, which had free posterior margins and obvious posterior

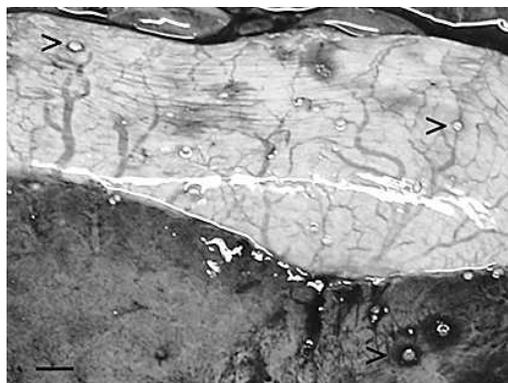


FIGURE 2. Obothrioid cestode cysts (several denoted by arrowheads) on serosal surface of stomach and liver of a Kemp's ridley sea turtle. Bar=10 mm.

bothrial pits. The tentacle sheaths were coiled, and there were no prebulbar organs; however, one or two gland cells were present near the base of the short oval bulbs (0.10 mm in length  $\times$  0.06 mm in width), which extended to the posterior margin and were not deviated. There were no glands in the rhynchocoel. The metabasal armature was heteroacanthus with predominantly unicate hooks and some falciform and spiniform hooks at the base with no basal swelling.

Single postlarval trypanorhynch cestodes were found free in the coelomic cavity of two turtles (Fig. 4). Both were large, active, white, and opaque, preventing inspection of internal structures. The first was 7.1 mm in fixed retracted length with a maximum width of 1.8 mm, and it had four narrow bothria fused to the pedicle, creating longitudinal ridges in the anterior two thirds of the parasite, and it had four slender hooked tentacles extending at least 0.4 mm from the scolex that were 0.1 mm in width (Fig. 4A). The second postlarval trypanorhynch cestode had a scolex (8.1 mm in length  $\times$  4.2 mm in width) with two labial bothria that could form a muscular triangular shape, and it extended perpendicular to the peduncle but did not have a free posterior margin. Two sets of two short globoid (1.2 mm in length  $\times$  0.8 mm in width) tentacles with

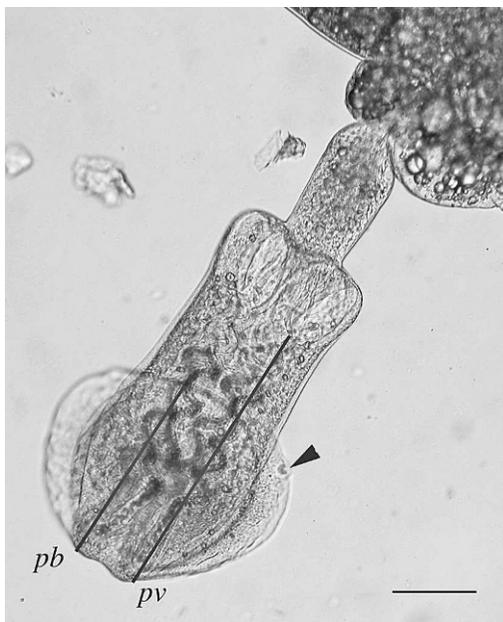


FIGURE 3. Obothrioid cestode cyst on the serosal surface of the small intestine of a Kemp's ridley sea turtle. Obothrioid plerocercus attached to blastocyst. pb = pars bothridialis; pv = pars vaginalis; arrowhead = bothrial pit. Bar=0.1 mm.

slender hooks protruded from between the bothria. The total fixed length was 28.3 mm with a maximal width of 7.3 mm, but the living form was distensible. The

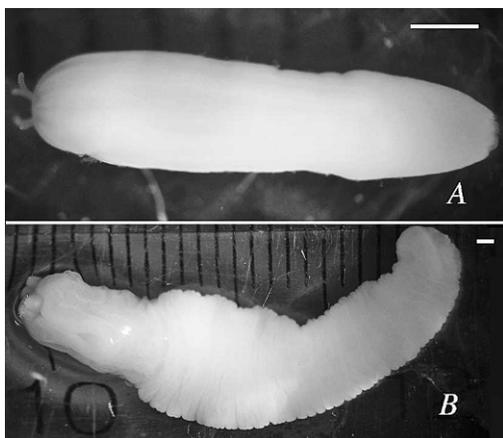


FIGURE 4. A. Postlarval trypanorhynch cestode (presumptive *Tentacularia coryphaenae*) from the coelomic cavity of a Kemp's ridley sea turtle. Bar=1 mm. B. Postlarval trypanorhynch cestode (presumptive *Hepatoxylon trichiuri*) from the coelomic cavity of a Kemp's ridley sea turtle. Bar=1 mm.

TABLE 1. Organ location and histopathologic lesions in 28 cold-stunned Kemp's ridley sea turtles (Abs = abscess; AMD = acute myofiber degeneration; B = bacterial; Bleph = blepharitis; C = cestodiasis; CL = cestode larva; Conj = conjunctivitis; D = dermatitis; Endo = endocarditis; Ent = enteritis; Esoph = esophagus; F = fungal; Gast = gastritis; Grm = granuloma; Hem = hemorrhage; IHM = intracorneal heterophilic microabscess; KS = keratoscleritis; L = lipidosis; LD = lymphoid depletion; MGP = meningeal granulomatous plaque; Micro = microsporean cysts; MU = mucosal ulceration; Myc = superficial epidermal mycosis; Myo = myositis; N = nematode; Neph = nephritis; P = parasite (not identifiable by histologic section); Panc = pancreatitis; PH = perihepatitis; Pl = pleural; PL = pleuritis; Pneum = pneumonia; Ser = serositis; T = trematodiasis; TN = tubular necrosis).

Turtle	Intestine	Stomach	Esoph	Liver	Pancreas	Lung	Brain	Eye	Skin	Eyelid	Shell	Kidney	Bladder	Ovary	Heart	Mesentery	Thymus	Muscle
1		Grm-CL		L, Grm-CL		Pl-Grm-F; Pl-Cyst-CL	KS				Micro	Grm-CL	Grm-CL					AMD
2			L			Pneum-F								Endo				
3			L															
4	C, T				Fibrosis													
5						GRN												
6		Grm-N									TN				Grm-N	Abs-B		
7		Grm	Grm	PH			Conj-B											Myo
8	Grm-CL					Pneum-F; Grm-N								Grm				
9		Grm				Pneum					TN				Myo			
10							Grm		Myo		TN							
11	Grm-CL					Pneum-F; Grm-N	MGP			IHM						Grm-P		LD
12	Abs-B, P, Grm-P						MGP			Bleph			Grm-P					
13	Grb-N			Grm-N														
14	Ent-B																	
15				Grm			MGP		Myo	IHM								
16	Grm-P					Pneum	MGP											
17	Ent-B						MGP											
18						Pneum												
19	Abs-B-P, Grm					PL	MGP		D, Myc				Ser					LD
20	Grm-P	Grm				Pneum-B												
21	Grm	Grm-N				Hem												
22	Grm-N				Panc													Grm
23		Gast-B				Pneum-F												

TABLE 1. Continued.

Turtle	Intestine	Stomach	Esoph	Liver	Pancreas	Lung	Brain	Eye	Skin	Eyelid	Shell	Kidney	Bladder	Ovary	Heart	Mesentery	Thymus	Muscle	
24		Gast-B, Gm				Pl-Pneum													
25		Gm				Pneum-F													
26	C	Gm-N	L																
27	Gm-CL	Gm-N																	
Total	14	11	1	7	2	16	9	2	6	5	1	5	3	2	4	3	2	2	2

body was ridged with horizontal folds (Fig. 4B).

**Pathology**

Lesions were most often identified in tissues of the alimentary (e.g., esophagus, stomach, intestine, liver, and pancreas) and respiratory (e.g., lung) systems, followed by the integumentary (e.g., skin, eyelids, and shell), nervous and sensory (e.g., meninges and eye), and urogenital (e.g., kidney, urinary bladder, and ovary) systems. Of the remaining tissues typically examined, few and generally mild or minimal lesions were identified in the heart, mesentery, thymus, and muscle. The anatomic distributions and tally of histopathologic lesions identified in each turtle necropsied during the study period are shown in Table 1.

**Alimentary system**

Twenty of 28 (71%) turtles had lesions in the digestive tract. Fourteen turtles had one or more lesions in the intestine, 11 had lesions in the stomach, and four turtles had lesions in both the intestine and stomach. One turtle had a lesion in the esophagus as well as one in the stomach. Mild to moderate gastritis, enteritis, or both were seen grossly in seven turtles. On gross examination, two turtles had solitary third stage larval nematodes (*Anisakis* sp.) in the serosal surface and muscularis of the small intestine. Identification of these larvae was based on morphologic characteristics, including the presence of three lips, larval tooth, mucron, simple esophagus and ventriculus, with no intestinal cecum (Davey, 1971; Smith and Wooten, 1978).

Histologic lesions of the digestive tract consisted predominantly of granulomas, often parasitic, involving the mural components of the esophagus, stomach, and intestine and generally were minimal or mild. Two specimens, however, had digestive tract lesions that were more severe and consisted of heterophilic abscesses and heterophilic granulomas with bacteria

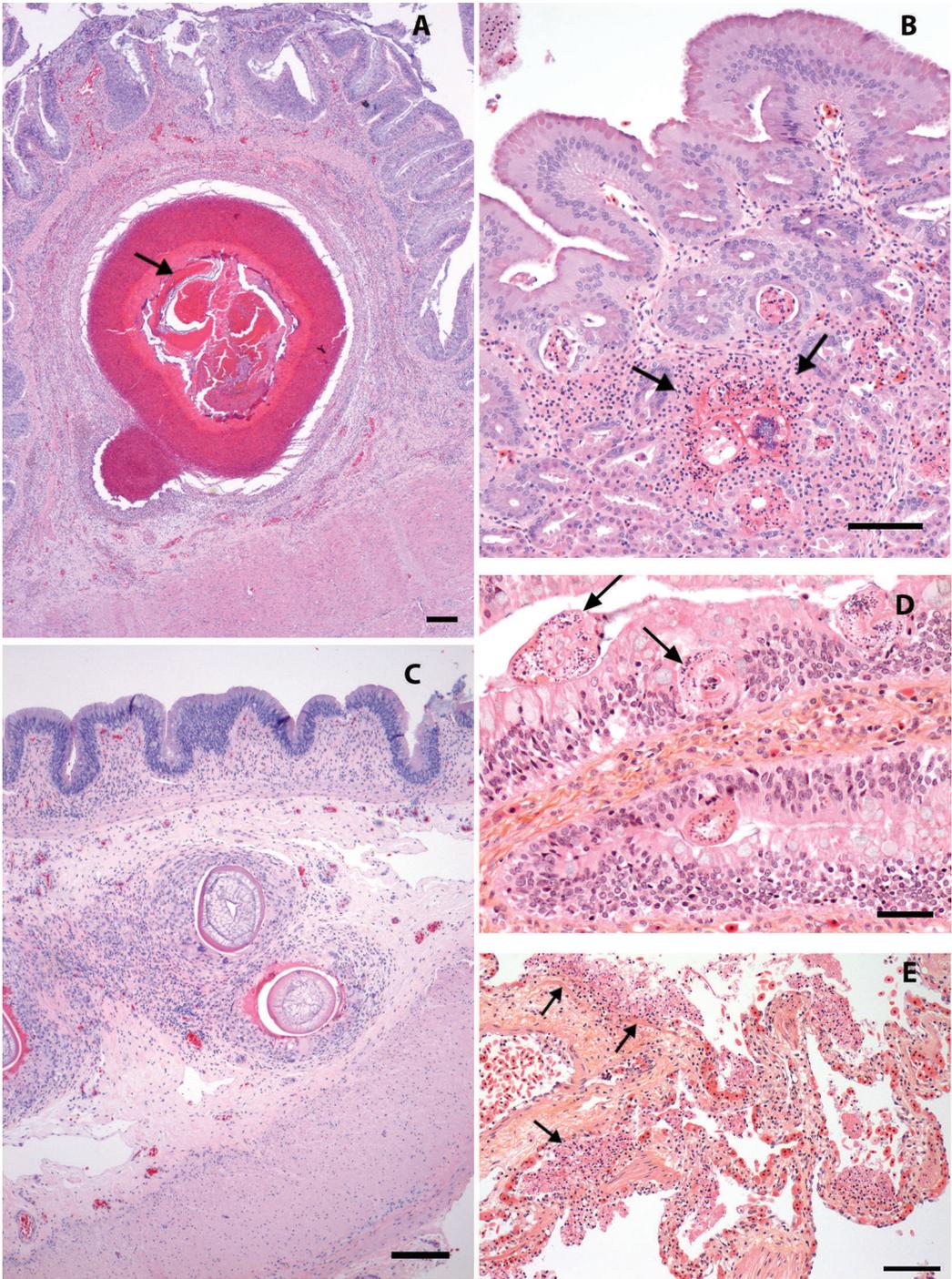


FIGURE 5. A. A heterophilic abscess expands the submucosa of the intestine. In the necrotic heterophilic debris of the abscess is the remnant of a nematode (arrow). Hematoxylin and eosin. Bar=200  $\mu$ m. B. An infiltrate of heterophils is present in the lamina propria (arrows) of the gastric mucosa and surrounds several necrotic glands, one of which contains an aggregate of bacteria. Other glands in the vicinity contain aggregates of heterophils in their lumina. Hematoxylin and eosin. Bar=100  $\mu$ m. C. Transverse sections of nematodes are located at the centers of granulomas in the submucosa of this section of intestine. Hematoxylin and eosin.

and remnants of metazoan parasites in the submucosa of the intestine (Fig. 5A). A marked, necrotizing, ulcerative and heterophilic enteritis, interpreted as subacute in duration, was identified in two specimens in association with gram-positive and gram-negative bacteria, whereas intestinal mucosal ulceration was identified in another. An acute, mild, focal gastritis, which was characterized by necrosis of few glands containing gram-negative bacteria together with lamina propria infiltrates of small to moderate numbers of heterophils, was identified in three other specimens (Fig. 5B). Granulomas were identified in the wall of the esophagus (one specimen), stomach (10 specimens), and intestine (10 specimens), and they had evidence of helminth parasites in multiple instances (Fig. 5C). Gastric parasitic granulomas included those that contained nematodes (four specimens) and larval cestodes (one specimen). Parasitic granulomas on the serosal surface of the intestine more commonly contained larval cestodes (three specimens), although one had nematodes. There were granulomas for which a causative agent was not identified in routine sections. Intestinal cestodiasis was identified in two specimens (Fig. 5D). Parasitic granulomas were identified in the mesentery of two turtles, and there was a heterophilic abscess in the mesentery of a third, in which gram-negative bacteria were identified.

With the exception of small numbers of superficial cestode cysts, the liver and pancreas were grossly normal in all cases. Histopathologic lesions were seen in the liver of seven cases. Three turtles had evidence of parasitic granulomas in either

the capsule (two specimens) or parenchyma (one specimen), which were associated with larval cestodes or nematodes, respectively. There was mild to moderate hepatic lipidosis in four specimens. One turtle had a mild, focal, granulomatous perihepatitis and capsular fibrosis for which an etiologic agent was not identified. Histopathologic abnormalities were seen in the pancreas in two cases. The lesion in one case was mild, focal and chronic, consisting of intralobular, interstitial fibrosis, whereas the lesion in the second case was marked and acute, consisting of heterophilic pancreatitis with hemorrhage and vasculitis.

#### Respiratory system

Sixteen turtles had lesions in the lung, pleura, or both. Gross respiratory tract lesions were identified in seven turtles and ranged from granulomas to locally extensive caseous exudates. Gross lesions were present in only the left lung of three specimens, only the right lung of two specimens, and bilaterally in two specimens. In seven turtles, there was white froth in the bronchi and lungs. The most frequently encountered histopathologic lesion was pneumonia, which was identified in 11 specimens. In eight specimens, the pneumonia was characterized as heterophilic and exudative, which was minimal to mild, acute and focal in four cases (Fig. 5E) and moderate or marked, acute or subacute, and multifocal in four other cases. There was involvement of the pleura in one of these cases (i.e., heterophilic and exudative pleuropneumonia), and a focal, necrotizing, and heterophilic pleuritis was identified in one other specimen. Acute heterophilic interstitial pneumonia was identified in one case.

←

Bar=200  $\mu$ m. D. Several cestodes (arrows) are located along the surface or buried in the mucosal epithelium of this section of intestine. Note that collagen stains yellow with this staining technique. Hematoxylin, phloxine, and saffron. Bar=50  $\mu$ m. E. Degenerate heterophils, protein, and necrotic debris line segments of faveolar septae, replacing the pneumocytic epithelium and capillaries (arrows), whereas erythrocytes and clumps of necrotic debris are present in faveolar spaces. Note that collagen stains yellow with this staining technique. Hematoxylin, phloxine, and saffron. Bar=200  $\mu$ m.

Chronic pneumonic lesions consisted of severe, focal, granulomatous pneumonia or heterophilic granuloma formation, which were identified in two specimens. Five cases of pneumonia were mycotic, wherein a hyalohyphomycete was identified and included mild, acute, heterophilic, and exudative; marked to severe, subacute, heterophilic, and exudative (Fig. 6A); and chronic, granulomatous forms. Two cases of acute heterophilic and exudative pneumonia were bacterial, and gram-negative bacteria were identified in these two instances. Etiologic agents were not identified in other instances of pneumonia. Granulomas were detected in the pleura of four specimens and contained nematodes or fungal hyphae in instances when etiologic agents were discernible, whereas encysted larval cestodes were identified in the pleura of one specimen.

#### **Nervous system**

No gross lesions were identified upon examination of the brain. Histopathologic lesions detected in brain consisted of minimal to mild, focal, plaque-like, granulomatous infiltrates in the meninges of eight specimens, in two of which there was mineralization (Fig. 6B). There was a minimal histiocytic granuloma (microgranuloma) in the neuropil of one specimen.

Histopathologic lesions were detected in the eyes of two specimens. In one specimen, a chronic keratitis with focal necrotizing and heterophilic scleritis was identified. In the second specimen, a mild, focal, necrotizing, and heterophilic bacterial conjunctivitis was noted.

#### **Urogenital system**

No gross urinary tract lesions were observed. Histopathologic lesions were identified in the kidney of four specimens. Three turtles had mild, acute, renal tubular degeneration and necrosis, whereas one specimen had marked, heterophilic, tubulointerstitial nephritis with gram-negative bacteria, which was interpreted as acute to subacute in duration. Few microsporidian

cysts adherent to the renal capsule were identified in one other specimen. Histopathologic lesions were seen in the urinary bladder of three specimens. Two specimens had minimal, subserosal, granulomas, one of which contained a larval cestode, whereas the third specimen had moderate, focally extensive, heterophilic serositis. Granulomas were identified in the ovary of two specimens, one of which contained a larval cestode.

#### **Integumentary and skeletal systems**

Ten turtles had full thickness defects of the keratin of the plastron and carapace, ranging from 1.0 cm to 3.0 cm in longest dimension, with ecchymoses of the underlying bone. Seven turtles had multiple abrasions, ulcerations, or lacerations of the skin of the head, eyelids, neck, or flippers, ranging from 0.5 cm to 5.0 cm in longest dimension. Five turtles had abrasions of the plastron and carapace that ranged from 0.5 cm to 3.0 cm in longest dimension. One turtle had a comminuted fracture involving approximately 20% of its anterior carapace and plastron, together with transection of the spinal cord, exposure of the coelomic viscera, and partial evisceration. Five turtles had minimal to moderate, superficial epidermal mycoses of the head or appendages, whereas one turtle had a mild epidermal mycosis of the carapace. Intracorneal heterophilic microabscesses were identified in the skin of one turtle, whereas necrotizing and ulcerative, heterophilic dermatitis was present in another. Histopathologic lesions were identified in eyelids of five specimens, two of which also had intracorneal heterophilic microabscesses with surface bacteria, whereas the other three had mild, focal, necrotizing and ulcerative, heterophilic blepharitis with bacteria (Fig. 6C).

#### **Cardiovascular system**

No gross lesions were observed in the heart or great vessels. Histopathologic lesions were seen in the heart of four

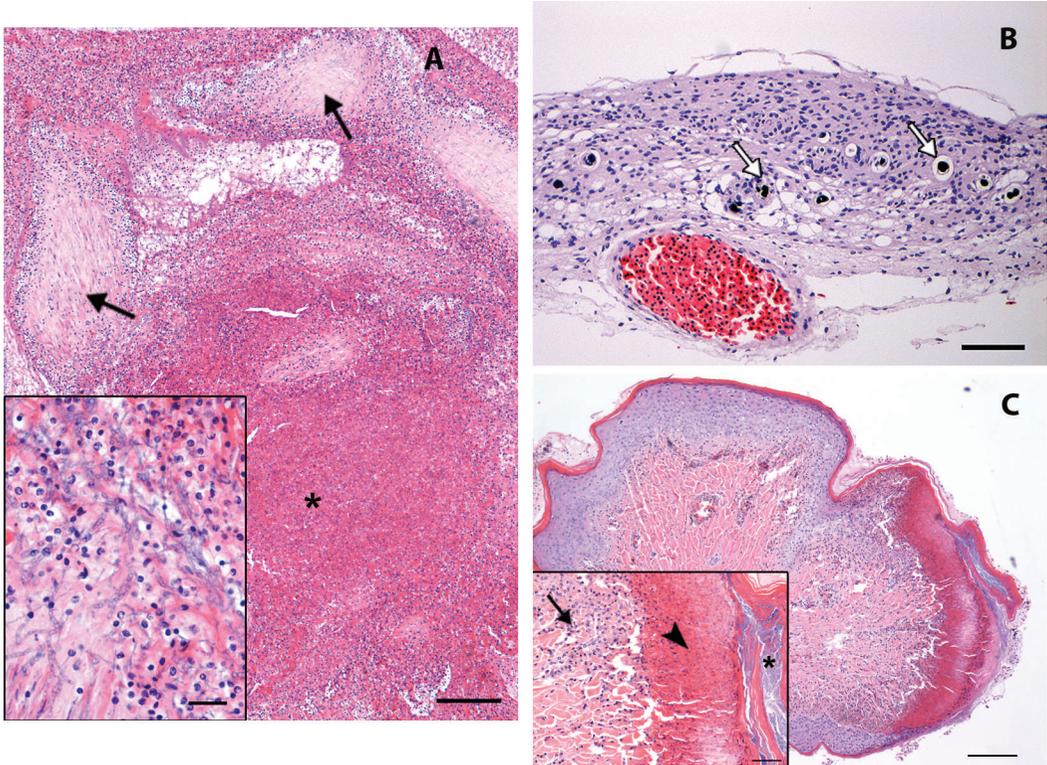


FIGURE 6. A. A focus of severe, heterophilic and exudative pneumonia is present and characterized by an extensive exudate of degenerate heterophils (\*) that fills the faveolar space and lines smooth muscle (arrows) of faveolar septae. Hematoxylin and eosin. Bar=200  $\mu$ m. Inset shows slender, filamentous fungal hyphae invade the interstitium and smooth muscle together with an inflammatory cell infiltrate. Hematoxylin and eosin. Bar=25  $\mu$ m. B. There is a plaque-like infiltrate of macrophages with deposits of mineral (arrows) in this segment of the meninges. Hematoxylin and eosin. Bar=100  $\mu$ m. C. A focus of blepharitis is present at the edge of the eyelid. Hematoxylin and eosin. Bar=200  $\mu$ m. Inset shows the dermis is infiltrated by heterophils (arrow) and the overlying epidermis is necrotic and replaced by a layer of densely packed degenerate heterophils (arrowhead). Bacteria are present between layers of keratin at the surface of the epidermis (asterisk). Hematoxylin and eosin. Bar=100  $\mu$ m.

cases and were minimal and focal. One turtle had histiocytic mural endocarditis. Another turtle had histiocytic myocarditis with myofiber degeneration and necrosis. Parasitic granulomas were identified in two other specimens (e.g., one a solitary myocardial granuloma with remnants of a nematode and the other an epicardial granuloma with the cuticular remnants of a helminth parasite).

#### Other tissues

Histopathologic abnormalities were seen in the thymus of two specimens.

Both cases had moderate to marked cortical lymphocyte depletion; in one specimen, there also were medullary heterophilic infiltrates. One turtle had mild, acute, multifocal skeletal myofiber degeneration and necrosis, whereas another had a minimal, focal, granulomatous myositis for which an etiologic agent was not identified.

No histopathologic lesions were identified in the following tissues: peripheral nerve, pineal gland, trachea, pulmonary artery and aorta, thyroid gland, salt gland, long bones, larynx, spinal cord, articular cartilage, oviduct, and testes.

## DISCUSSION

Although rescue and rehabilitation efforts for cold-stunned sea turtles have been relatively well documented over the past 15 yr, causes of death for cold-stunned turtles have not been completely elucidated. This report provides detailed descriptions of pathologic findings in 28 cold-stunned Kemp's ridley sea turtles over a 6-yr period, which provides data from a much larger number of turtles over a longer time than previous reports (Matassa et al., 1994; Sadove et al., 1998; Smith et al., 2000; Turnbull et al., 2000; Harms et al., 2002; Manire et al., 2002; Wyneken et al., 2006). Additionally, this study is the first to exclusively describe pathologic findings that were present at the time of stranding, rather than later in the rehabilitation process. Lesions were minimal to mild in 50% of the turtles and in many cases could be considered incidental, suggesting that factors other than organ-specific disease or systemic infection were likely involved in the death of these turtles. Although some sea turtles may be prone to cold-stunning due to pre-existing pathologic conditions, many turtles with minimal lesions were also affected.

Among those turtles that did have significant pathologic changes, findings were consistent with previous reports on smaller numbers of cold-stunned turtles (Matassa et al., 1994; Sadove et al., 1998; Smith et al., 2000; Turnbull et al., 2000; Harms et al., 2002; Manire et al., 2002; Wyneken et al., 2006). Lesions of the respiratory, digestive, integumentary, nervous, and urinary systems, as seen in cold-stunned Kemp's ridley turtles, have also been reported in several reviews of pathologic findings of sea turtles from around the world (Glazebrook and Campbell, 1990; Orós et al., 2004, 2005; Jacobson et al., 2006). In many cases, pathologic changes in the cold-stunned turtles' tissues were associated with bacterial and fungal organisms. It is generally accepted that bacterial and fungal diseases

of reptiles are often the result of opportunistic infection of an immunocompromised host, and exposure to environmental temperatures below the optimum temperature range of the host species is a common cause of immunosuppression (Rosenthal and Mader, 2006). It is likely that impairment of the immune response due to prolonged hypothermia is partly responsible for the bacterial and fungal infections seen in cold-stunned Kemp's ridley sea turtles.

The initial cell in acute inflammatory reactions in reptiles is the heterophil (Montali, 1988; Stacy and Pessier, 2007), and inflammatory infiltrates in the turtles of this study that were predominantly heterophilic were interpreted as acute or subacute. Interpretations of the temporality of inflammatory infiltrates in reptiles are difficult, however, because inflammatory responses are profoundly influenced by environmental temperature (Stacy and Pessier, 2007). Studies of the effect of ambient temperature on wound healing in the common garter snake (*Thamnophis sirtalis*) indicate that the appearance of histopathologic features associated with healing and the related disappearance of those reflecting inflammation were more rapid in snakes held at higher temperatures, although the sequence of cellular events in wound healing was the same regardless of ambient temperature (Smith et al., 1988). One would expect, therefore, that the nature of cellular events and histopathologic features in inflammatory responses of cold-stunned sea turtles would be the same as those of sea turtles initiated within the voluntary temperature range of the species (i.e., the thermal range within which the animal is voluntarily active; Smith et al., 1988). Heterophilic exudates identified in instances of pneumonia in these sea turtles represent early inflammatory responses. In birds with early pulmonary aspergillosis, lesions that begin as focal accumulations of heterophils in bronchi and capillary air spaces can progress to granuloma forma-

tion in a week or less (Montali, 1988). Irritant-induced heterophilic infiltrates in alligators were followed within 24 hr by monocytes, and heterophilic granuloma formation occurred as early as 7 days (Mateo et al., 1984). Similarly, heterophils and macrophages were observed within 2 days in surgically produced wounds in common garter snakes (Smith and Barker, 1988). These previous reports would suggest that the heterophilic and exudative pneumonic lesions observed in turtles of this study could have formed days before death in contrast to the granulomas and granulomatous forms of pneumonia observed, which may have developed a week or more before death. However, in reality, such an interpretation requires an immediate disclaimer because neither the duration of hypothermia nor its subsequent influence on the magnitude of the delay in the development and maturation of the inflammatory response was either predictable or consistent from one cold-stunned turtle to the next.

This is the first report of helminth parasites from Kemp's ridley turtles. Trypanorhynch cestodes were identified by simple morphologic measurements, readily observable features, and by exclusion, following the descriptions of Palm (2004). The trypanorhynch plerocercoids freed from their blastocysts on serosal surfaces were assigned to the superfamily Otobothrioidea based on the following characteristics: elongated scolex, free lateral and posterior margins of the bothria, posterior bothrial pits, and the absence of prebulbar organs or glands within the bulbs. Within the Otobothrioidea, the serosal trypanorhynch cestodes did not belong to the family Paranybeliniidae because the pars vaginalis of the plerocercoid was longer than the pars bothrialis. The serosal cestodes also did not fit the description of any of the five species in the family Pseudotobothriidae: *Parotobothrium balli* has lateral not posterior bothrial pits, and *P. dollfusi* has predominantly falciform not unicate hooks. *Pseu-*

*dotobothrium dipsacum* has thick bothrial margins and elongate bulbs that diverge laterally, and longitudinal muscles not seen in our specimens. *Pseudotobothrium arii* and *Poeciloacanthum oweni* both have banana-shaped bulbs that diverge laterally. All five species are also two to 11× longer (scolex length) than our specimens. Based on the described morphologic characteristics, the serosal trypanorhynch plerocercoids of the Kemp's ridley turtles were of the family Otobothriidae. However, a detailed study of the tentacle armature was not performed, which would be required to identify the organisms to the genus level. *Otobothrium cysticum* has been reported from the serosal surface of the intestine of green sea turtles (*Chelonia mydas*; Mayer, 1842), and although our specimens were consistent with descriptions of *O. cysticum*, recent studies have determined this species to be of questionable validity (Beveridge and Justine, 2007).

The trypanorhynch postlarvae were both white and opaque, and the internal structures were not observed. The ratio of the pars vaginalis to the pars bothrialis could not be determined. The large scolices and fused bothria of the postlarvae were characteristic of the superfamily Tentacularioidea, where only six of the 58 species were comparable in size to our specimens (Palm, 2004). The first postlarval cestode (Fig. 4A) had four elongated bothria completely fused to the peduncle, no bothrial pits, and four thin tentacles, which were characteristic of the monotypic genera *Tentacularia*, *Kotorella*, or *Kotorelliella*. Species of the latter two genera are smaller than our specimen (Palm, 2004). Therefore, our specimen was most consistent with *Tentacularia coryphaenae*, which has been described from the green sea turtle and loggerhead sea turtle (*Caretta caretta*; Dollfus, 1942; Dodd, 1988) and is generally considered cosmopolitan (Palm et al., 2007). The second trypanorhynch postlarval cestode (Fig. 4B) had two bothria,

characteristic of the family Sphyricephalidae (Palm, 2004). The morphology of the tentacles of our specimen was characteristic of the genus *Hepatoxylon*, which includes two species. *Hepatoxylon megacephalum* has longer, conical, and thinner tentacles than *H. trichiuri*, which has shorter, globoid tentacles (Palm, 2004). Thus, our specimen was most similar to *H. trichiuri*. This species has not been reported from reptiles but has more than 90 fish species as intermediate hosts. Adult worms of this species live in large pelagic sharks (Palm, 2004).

Tetraphyllidean cestodes in any developmental stage have not been reported previously in sea turtles, but tetraphyllidean plerocercoids are commonly seen in the intestines of teleost fish and have also been reported from dolphins (Agusti et al., 2005). In the Kemp's ridley turtles, these cestodes were confined to the intestinal lumen and mucus layer and were not associated with pathology. Thus, it is possible that they were passing through the gastrointestinal tract without establishing infection.

Species of *Anisakis* nematodes are common parasites of marine mammals. Larvae of *Anisakis* sp. have been reported from the stomach of green sea turtles and loggerhead sea turtles (Burke and Rogers, 1982; Glazebrook and Campbell, 1990; Piccolo and Manfredi, 2003). In some cases, these larvae have been associated with gastric ulceration, whereas in other cases no pathology was present (Burke and Rogers, 1982; Glazebrook and Campbell, 1990; Piccolo and Manfredi, 2003). In this study, microscopic examination of tissues from several specimens revealed nematode-containing granulomas; however, lesions were generally limited in number and mild to moderate in severity. Definitive species identification of the species of *Anisakis* requires morphologic examination of adult male worms, culture, or molecular methods. Although beyond the scope of this study, future studies of the cestode and nematode parasites of

Kemp's ridley turtles should include scanning electron microscopy, *in vitro* culture, and molecular techniques for more definitive species identification.

In contrast to many previous reports, stranded turtles in this study had a very low incidence of significant lesions caused by human interaction or parasitism, and no turtles were affected by fibropapillomatosis (Glazebrook et al., 1990; Gordon et al., 1993; Orós et al., 2004, 2005; Foley et al., 2005; Jacobson et al., 2006). In only one case, the turtle with a severe carapace fracture, was a traumatic injury likely caused by human interaction (e.g., boat strike). In most cases, skin and shell abrasions were likely caused during the stranding process as the turtles were washed onto the beach. Sea gulls have been observed to predate stranded sea turtles and could also have caused some of the noted external injuries (Merigo, pers. obs.).

The etiology of the granulomatous infiltrates seen in the meninges of several turtles in this study is unknown. The most common cause of central nervous system granulomas in sea turtles is spirorchid trematode infection (Jacobson et al., 2006); however, it is interesting to note that no trematodes or trematode ova were seen grossly or histologically in any tissues in these cold-stunned turtles. Although gross and histologic examination can often detect spirorchid trematodes and their ova, more sensitive detection methods have been described previously (Snyder and Clopton, 2005). Such methods should be considered for future parasitologic investigations of cold-stunned Kemp's ridley sea turtles.

Many turtles had little, if any, ingesta in the digestive tract. It is unknown whether this reflects anorexia due to illness or may be due to seasonal fasting. Among those turtles in which ingesta could be identified, the spectrum of food items is consistent with previous reports that characterize juvenile Kemp's ridley turtles as benthic foragers (Burke et al., 1993). The algae, barnacles, and slipper shells

noted on the shell of several turtles are commonly seen on sea turtles, and in no case were the epibiota associated with noticeable lesions, except for mild disruption of the keratin at the point of attachment. Species of barnacles reported previously on the Kemp's ridley turtle include *Stomatolepas praequestator*, *Platylepas hexastilos*, *Chelonibia testudinaria*, and *Balanus amphitrite* (Marquez-M., 1994). The presence of *B. venustus* in several turtles in this study represents a new host record for this species.

In Massachusetts, cold-stun events typically occur in November and December when water temperatures drop below 12 C (Still et al., 2005). Although the term "hypothermia" may not be completely appropriate to use for an ectothermic species, it is possible that cold-stunned turtles suffer from similar pathophysiological events as those seen in mammals under conditions of hypothermia. In human forensic pathology, the diagnosis of hypothermia as cause of death is a diagnosis of exclusion and circumstantial evidence (Lifschulz and Donoghue, 1998; Nixdorf-Miller et al., 2006). Some of the histopathologic findings in the turtles of this report may be consistent with lesions seen in human cases of hypothermia. For example, gastric or duodenal submucosal bleeding and erosions may be seen in human hypothermia deaths, and similar lesions were seen in several turtles in this study (Hirvonen, 1976; Mizukami et al., 1999).

It is also possible that drowning or near drowning may contribute to morbidity and mortality of cold-stunned sea turtles. It is likely that weak, poorly responsive turtles may have difficulty surfacing to breathe and may be prone to aspiration of sea water. In human forensic pathology, the diagnosis of drowning, like that of hypothermia, is not straightforward, and gross autopsy and histopathologic findings are often nonspecific (Piette and DeLetter, 2005). Some support for drowning or near drowning as a factor in sea turtle mortality is found in several studies that report

hypermagnesemia in stranded sea turtles (Turnbull et al., 2000; Wyneken et al., 2006; Innis et al., 2007). Hypermagnesemia has also been reported in humans that have drowned in sea water, presumably due to aspiration of the magnesium-rich water (Zhu et al., 2005). Bacterial and fungal pneumonia have been reported as sequelae to near-drowning in humans (Janssen et al., 1996; Miyake et al., 2000). Whether aspiration of sea water is directly related to the relatively high incidence of pneumonia seen in cold-stunned Kemp's ridley turtles is open to speculation. However, factors such as hypothermia and aspiration of sea water, either alone or in combination, that might impair function of the mucociliary apparatus of the respiratory tract could predispose turtles to bacterial or fungal pneumonia.

Clinical pathologic findings and physical examination data from cold-stunned Kemp's ridley sea turtles indicate that many turtles are affected by dehydration, metabolic and respiratory acidosis, bradycardia, apnea, and severe electrolyte imbalances, such as hyperkalemia (Turnbull et al., 2000; Wyneken et al., 2006; Innis et al., 2007). Hence, the cause of death for cold-stunned sea turtles is likely multifactorial, including metabolic and respiratory derangements, pathophysiological effects of hypothermia, and drowning/near-drowning, with or without additional organ-specific lesions.

This study provides insight into the variety of pathologic and parasitologic findings that may be found in cold-stunned Kemp's ridley sea turtles at the time of stranding. These results provide clinically relevant information that may aid in rehabilitation efforts. Specifically, clinical management of cold-stunned turtles should include careful evaluation of the respiratory, digestive, integumentary, nervous, and urinary systems. Identification of lesions early in the course of hospitalization may increase the likelihood of successful rehabilitation.

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