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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Pathological Findings in Leatherback Sea Turtles (*Dermochelys coriacea*) During an Unusual Mortality Event in São Paulo, Brazil, in 2016

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Summary

The leatherback sea turtle (LST; *Dermochelys coriacea*) is highly migratory and is primarily distributed throughout tropical and subtropical regions. The LST populations found in Brazil are critically endangered. An unusual mortality event occurred between August and November 2016 with 23 LSTs stranded along a 100 km coastal segment in Iguape, Ilha Comprida and Ilha do Cardoso in southern São Paulo state. This study investigated the pathological findings and most likely causes of death of 10 LSTs. Male (n = 9) and adult (n = 9) animals predominated. All but one animal was in good body condition and all were found dead. The most prevalent gross findings were suggestive of bycatch, namely cutaneous erosions, abrasions and/or lacerations around the neck and flippers (n = 9), generalized congestion (n = 8) and pulmonary oedema (n = 6). Other prevalent gross findings were: cutaneous epibiosis by *Stomatolepas elegans* (n = 7); ileocecal diverticulitis (n = 7); distal oesophagitis (n = 5); and fibrinous coelomitis (n = 5). Microscopically, the most prevalent findings were: hepatic melanomacrophage centre hypertrophy or hyperplasia (n = 9); interstitial pneumonia (n = 8); multisystemic congestion (n = 6); pulmonary oedema with or without aspirated material (n = 5); adrenal coccidiosis (n = 5) with variable multiorgan involvement; and multiorgan bacterial disease (n = 5). Five animals had food ingesta (cnidarians, crustaceans) in the oesophagus or stomach; only one had evident plastic foreign bodies. Asphyxia due to entanglement in nets was the most frequently identified cause of death (n = 8); a cause of death was not identified in two animals. Our findings provide evidence of the severe negative impact of entanglement in fishing nets in LSTs, raising concerns for conservation. These findings also contribute to knowledge of the pathology of LSTs in South American populations.

Keywords: adrenal coccidiosis; bycatch; pathology; sea turtles

Marine turtles, encompassing seven extant species, are sentinels of ecosystem health (Aguirre and Lutz, 2004). The leatherback sea turtle (LST; *Dermochelys coriacea*) is the largest living species and is primarily distributed throughout tropical and subtropical regions with seven subpopulations recognized in distinct regional management units (RMUs) (Wallace et al., 2010). LSTs often perform transoceanic migrations between reproductive and foraging zones (Benson *et al.*, 2007). In Brazil, LSTs inhabit the South Atlantic (Southwest and Southeast) RMU. From one to 19 female LSTs nest in Brazil each year, typically in the State of Espírito Santo (lat. 18.3°–21.2°S) with occasional nesting elsewhere along the Brazilian coast, including southern São Paulo state (Thomé *et al.*, 2007; Almeida *et al.*, 2011).

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The LST is globally classified as vulnerable by the International Union for Conservation of Nature 'IUCN' (Tiwari *et al*., 2013). However, the Southwest Atlantic leatherback population (SWA-LST) is classified as 'critically endangered' by the IUCN and listed on the national registry of endangered species (Almeida *et al*., 2011; Tiwari *et al*., 2013). Anthropogenic activities are the leading causes of declining sea turtle populations worldwide (Wallace *et al*., 2011). Currently, uncontrolled expansion of fishing operations is considered the main threat to sea turtles in Brazilian waters, but other direct and indirect anthropogenic factors also impact these species (Harvell, 1999; Reis *et al*., 2010; Almeida *et al*., 2011). Natural threats, mainly infectious and parasitic diseases, are causes of significant morbidity and mortality in sea turtles (Alfaro *et al*., 2008; Poppi *et al*., 2012; Miller *et al*., 2013).

Despite a few recent studies dealing with health assessments in free-ranging LSTs, health information on this species remains comparatively limited (Innis *et al*., 2010; Poppi *et al*., 2012; Miller *et al*., 2013; Stacy *et al*., 2015; Ferguson *et al*., 2016; Hill *et al*., 2019). The difficulty in accessing fresh carcasses of this large pelagic species largely accounts for the current lack of knowledge on its physiology and biology and the potential negative impact of anthropogenic factors and the recognition of emerging diseases (Innis *et al*., 2010).

From September to November 2016, an unusual mortality event (UME) involving 23 LSTs occurred in the southern coast of São Paulo state, involving the Iguape-Cananéia-Paranaguá estuary lagoon complex. This area is considered an important sea turtle feeding ground, with strandings and sightings year-round of the five sea turtle species that occur along the Brazilian coast (Guebert-Bartholo *et al*., 2011). Strandings of LSTs for the equivalent period in previous years ranged from 0 to 5 specimens (SIMBA, 2020). This study investigated the pathological findings and most likely causes of death of 10 LSTs that died in that UME.

The study area comprised approximately 100 km of coastal waters (25.3045885°S; 48.096436°W–24.573911°S; 47.245427°W) along three islands: Iguape, Ilha Comprida and Ilha do Cardoso (Cananéia; São Paulo state). Since August 2015, daily beach surveys have been conducted by Instituto de Pesquisas Cananéia, which is affiliated to the Beaches Monitoring Program of the Bacia de Santos (PMP-BS). Carcasses were classified as fresh or with moderate, advanced or very advanced autolysis according to PMP protocol (Table 1). Carcasses with very advanced autolysis and those with no graphic records, incomplete necropsy or lack of histopathological examination were excluded from the study. Evaluated turtles were classified into two age classes according to the curved carapace length (measured from the middle of the nuchal notch to the posterior-most tip of the caudal peduncle) (Thomé *et al*., 2007; Eckert *et al*., 2012). Body condition was subjectively classified as good, moderate or poor if coelomic and mesenteric fat deposits appeared abundant, moderate or sparse, respectively. Muscle mass in the pectoral, axillary and inguinal regions as well as plastron convexity were evaluated (Flint *et al*., 2009). The body condition index was not determined due to the lack of body weight. Stranding dates and biological data are recorded in Table 1.

Necropsies were performed according to published procedures (Flint *et al*., 2009; Work, 2014). Representative tissue samples of main organs (Supplementary Table 1) were collected and fixed in 10% neutral buffered formalin or frozen at −20 °C until analysis. The tissue samples were processed routinely through graded alcohols prior to being embedded in paraffin wax and 5 µm thick sections were stained with haematoxylin and eosin (HE) for microscopic analysis.

Table 1
Leatherback sea turtles (*D. coriacea*) included in this study. Body condition (BC); carcass condition (CC): fresh (2), moderate (3), advanced decomposition (4). Curved carapace length (CCL) in centimetres.

No	*Date*	*Sex*	*Age*	*CCL (cm)*	*BC*	*CC*	*Stranding history*
1	21/10/2016	Male	Adult/Subadult	148	Moderate	3	All LSTs were found
2	04/11/2016	Male	Adult/Subadult	153	Poor	2	dead during an UME
3	22/10/2016	Male	Juvenile	128	Moderate	4	between August and
4	15/10/2016	Male	Adult/Subadult	140	Good	3	November 2016 in the
5	23/09/2016	Male	Adult/Subadult	143	Moderate	3	southern coast of the state
6	28/08/2016	Male	Adult/Subadult	143	Moderate	3	of São Paulo, Brazil.
7	18/10/2016	Female	Adult/Subadult	139	Good	3	
8	25/10/2016	Male	Adult/Subadult	145	Moderate	4	
9	27/10/2016	Male	Adult/Subadult	144	Moderate	3	
10	23/10/2016	Male	Adult/Subadult	144	Moderate	3	

Additional histochemical stains used to better characterize the lesions in selected cases included periodic acid–Schiff (for fungi and basement membranes), Grocott-Gomori's methenamine silver (for fungi), Ziehl–Neelsen (for mycobacteria), Gram/Twort (for bacteria) and Masson's trichrome (for collagen/fibrosis).

One animal was juvenile (1/10; 10%) and nine were adult or subadult (9/10; 90%). The single female was classified as an adult. Sex determination was by gross gonadal visualization.

The most common gross findings were: erosions, abrasions and/or lacerations around the neck, flippers and carapace (9/10; 90%); cutaneous epibiosis by *Stomatolepas elegans* (9/10; 90%); generalized congestion and petechiae (8/10; 80%); ileocaecal diverticulitis (7/10; 70%); pulmonary oedema and congestion (6/10; 60%; Fig. 1); subcutaneous oedema (6/10; 60%); multifocal healed skin wounds (6/10; 60%); cutaneous erythema (5/10; 50%); hydropericardium (5/10; 50%); food ingesta (cnidarians, crustaceans) in the oesophagus and/or stomach (5/10; 50%) (Supplementary Table 1); numerous intra-atrial gas bubbles (4/10; 40%); distal oesophagitis (4/10; 40%); and polyserositis (4/10; 40%). Only one animal (1/10; 10%) had evident plastic foreign bodies (four pieces of small irregular soft plastic amid faeces). One turtle had a cutaneous fibroma in a hindlimb, presumably associated with historical tagging. Another had multiple amputated right anterior and posterior phalanges with soft tissue loss and marginal osseous calluses, highly suggestive of historical predatory shark attack.

Fig. 1. Pulmonary oedema and presumed aspirated seawater. Note the congested and oedematous pulmonary parenchyma on cut surface (asterisks; case 1).

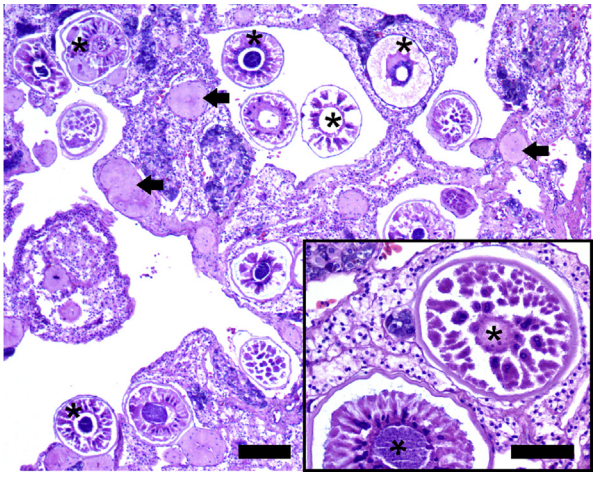

Fig. 2. Adrenal coccidiosis in a leatherback sea turtle (*D. coriacea*; case 6). Protozoan cysts (asterisks) and discrete fibrotic nodules (arrows) expand the adrenal trabeculae. HE. Bar, 200 μm. Inset: Detail of adrenal protozoan cysts Bar, 100 μm.

Microscopic lesions observed were: adrenal coccidiosis (6/6 [denominator indicates animals with organ examined]; 100%) (Fig. 2); marked ileocaecal diverticulitis (7/7; 100%); hepatic melanomacrophage centre hypertrophy/hyperplasia (9/9; 100%); mild, multifocal, chronic interstitial nephritis (7/7; 100%); mild, multifocal, chronic interstitial pneumonia (6/7; 86%); mild to moderate, multifocal, chronic oesophagitis (6/8; 75%); mild, multifocal, chronic omyocarditis (4/6; 67%); generalized congestion and haemorrhage (6/10; 60%), typically involving the thyroid glands (4/4; 100%), spleen (2/5; 40%), kidney (2/7; 29%), lung (2/7; 29%) and liver (2/9; 22%); marked pulmonary oedema with or without aspirated material (4/7; 57%); and one case with presumed cutaneous herpesvirus infection based on the presence of intranuclear inclusion bodies in keratinocytes. Coccidia were also noted in the urinary bladder (1/2; 50%), small intestine (2/7; 29%) and heart (1/6; 17%). Adrenal coccidiosis was characterized by mild to marked, multifocal, chronic pleocellular inflammatory infiltrates with protozoan cysts and frequent nodular fibrosis. The detailed pathological findings are recorded in Supplementary Table 1.

Based on the frequent occurrence of cutaneous erythema, erosions, abrasions and/or lacerations around the neck, flippers and carapace, pulmonary oedema and congestion, subcutaneous oedema, hydropericardium, generalized congestion and petechiae and intra-atrial gas bubbles, the most likely cause of death in these cases was considered to be entanglement in fishing nets (Work and Balazs, 2013; Work *et al.*, 2015; Domiciano *et al.*, 2017).

Cardiorespiratory collapse by asphyxia associated with bycatch was the most likely cause of death (CD) in 8/10 (80%) turtles. A CD was not apparent in 2/10 (20%) animals. These results suggest cardiorespiratory collapse by asphyxia as the leading CD of LSTs in the UME recorded in southern São Paulo State (Brazil), between August and November of 2016. A previous study suggested that these turtles might have approached the coast to forage on an unusual bloom of jellyfish in the area, possibly due to an increase of SE trade winds during the 'La Niña' season. These circumstances could have resulted in an increased risk of interaction with coastal fisheries (Nagaoka *et al*., 2018).

The bycatch-associated pathological findings in these turtles were largely similar to those described in other sea turtles species and included acute cutaneous trauma and acute haemodynamic alterations in multiple organs suggestive of shock, probably due to a combination of cardiogenic and neurogenic mechanisms (Work and Balazs, 2013; Work *et al*., 2015; Domiciano *et al*., 2017). Findings suggestive of potential in-vivo gas decompression phenomena during bycatch, namely intra-atrial gas bubbles (identified transmurally without incising the pericardium or myocardium), were similar to those reported in loggerhead sea turtles (*Caretta caretta*) bycaught in trawls and gillnets of local fisheries from the east coast of Spain (García-Párraga *et al*., 2014). As proposed for loggerhead sea turtles, intravascular gas bubbles may provide additional pathological evidence of fishing interactions in LSTs. In this case, intravascular gas bubbles together with external lesions of erythema, erosions, abrasions and lacerations around the neck, flippers and carapace and sand in the lower respiratory tract could indicate capture in a bottom-set or bottom-trawl net.

This study detected a high frequency of ileocaecal diverticulitis, suggesting that this lesion is a frequent finding in subadult and adult LSTs in Brazilian waters. These lesions were identical to those described in North America LST populations, characterized by a solitary exudate-filled diverticulum emanating from the region of the ileocaecal junction (Stacy *et al*., 2015). In none of our cases was there any evidence that diverticulitis was associated with death. The aetiopathogenesis and clinical-pathological significance of this finding remains unclear.

We observed adrenal coccidiosis in six animals with concomitant involvement of the urinary bladder, small intestine or heart in five turtles. Adrenal coccidiosis with identical histopathological features was recently recorded for the first time in North American LSTs (Ferguson *et al*., 2016). Based on molecular analyses of two 18S sequences from LSTs affected, the authors concluded that the adrenal coccidian belonged to the family Eimeriidae. In contrast to that report, the LSTs described in our study had coccidial infection in multiple organs including the small intestine, urinary bladder and heart. These findings suggest that this parasitic infection may not be restricted to the adrenal glands in this species, as previously thought. We conducted polimerase chain reaction (PCR) analyses in formalin-fixed and paraffin wax-embedded adrenal gland tissue; however, total deoxyribonucleic acid (DNA) extracted and amplified from these samples was insufficient for characterization purposes (data not shown). Therefore, no conclusions can be drawn regarding the identity of this histomorphologically identical coccidian. Although the potential clinical-pathological significance of these findings remains unclear, they were not considered to be associated with death in any of the turtles. Further molecular and pathological studies will focus on definitive identification, pathophysiology and potential health implications of this coccidian on the turtle host.

One LST presented a cutaneous fibroma in the left hindlimb, associated with a regular circular scar, most likely the result of tagging. Despite the fact that several tumour subtypes in LSTs have been linked to *Chelonid alphaherpesvirus* 5 (ChHV5) (Work *et al*., 2004; Díaz-Delgado *et al*., 2019), the PCR analyses performed in this specimen (data not shown) failed to detect ChHV5. Additional findings in these LSTs were deemed of minor clinical-pathological relevance. These included concomitant local or multiorgan discrete infectious inflammatory processes, typically with bacterial involvement. Bacterial infections, either primary or secondary, are common in sea turtles, often involving the gastrointestinal, respiratory and urinary systems (Mader, 1996; Orós *et al*., 2005; Jacobson, 2007; Alfaro *et al*., 2008).

In summary, these results indicate that trauma from fishing nets was a major contributor to the UME of LSTs in the south coast of São Paulo State. These findings are highly suggestive of bottom-set or bottom-trawl net interaction, raising concerns for conservation and the need for developing mitigation actions and political policies to reduce bycatch impacts on sea turtle populations. The first report of adrenal coccidiosis with multiorgan involvement and ileocaecal diverticulitis in LSTs in Brazilian waters is provided. These results enhance knowledge of the pathology of this species in this geographical area and may be of value to first responders, veterinarians and diagnosticians. They may also provide a scientific basis for future conservation efforts on LSTs.

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Conflict of Interest Statement

The authors declare no potential conflicts of interest with respect to the research, authorship or publication of this article.

Supplementary data

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