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Pansteatitis associated with high levels of polychlorinated biphenyls in a wild loggerhead sea turtle *Caretta caretta*

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ABSTRACT: We describe the morphologic and toxicological findings in a case of pansteatitis in a stranded loggerhead sea turtle *Caretta caretta*. At necropsy, a large amount of adipose tissue in the celomic cavity showing very firm, yellow to orange irregular formations was observed. Histological lesions ranged from the infiltration of necrotic fat by scarce multinucleated giant cells and numerous macrophages containing xylene-insoluble lipopigment inclusions to the presence of several granulomas characterized by an irregular central necrotic area consisting of lipopigment surrounded by numerous multinucleated giant cells. Microbiological cultures were negative. Celomic fat was analyzed for PCBs and DDTs, resulting in very high levels of PCB 138, 153, 180 209 (3170, 2830, 980 and 1190 ng g^{-1}, respectively). Although a nutritional cause cannot be ruled out, the high levels of PCBs detected in the celomic fat could have induced lipid peroxidation in adipocytes, resulting in cell damage, deposition of ceroid pigment and inflammatory response. This is the first report of pansteatitis in a wild sea turtle.

KEY WORDS: Contaminants · PCBs · DDTs · Disease · Lipid peroxidation

INTRODUCTION

All species of sea turtles are included in the Red List of the World Conservation Union (IUCN 2012). In recent years, increased efforts have been devoted to the conservation of sea turtles, including medical management and pathological studies on stranded animals (Work et al. 2004, Orós et al. 2005). Although the most common species in the Canary Islands is the loggerhead turtle, evidence of a decline in the population of loggerheads in the Canary Islands has been reported (Blanco & González 1992).

Pansteatitis describes generalized inflammation of the subcutaneous and intra-abdominal fat, mainly associated with vitamin E deficiency and with the same etiologies described for panniculitis (German et al. 2003). Among the class Reptilia, pansteatitis has only been described in captive American alligators *Alligator mississippiensis* (Larsen et al. 1983), farmed (Huchzermeyer 2003) and wild Nile crocodiles *Crocodylus niloticus* (Bouwman et al. 2009), and captive olive ridley sea turtles *Lepidochelys olivacea* (Manawatthana & Kasorndorkbua 2005).

Although there are numerous studies reporting baseline levels of contaminants in several species of sea turtles from different geographical areas, available data on the effects of these contaminants on diseases and lesions of sea turtles are scarce

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(Keller et al. 2004b, Swarthout et al. 2010, Orós et al. 2012).

Since 1994, the Veterinary Faculty at the University of Las Palmas de Gran Canaria (ULPGC) has been carrying out a survey of lesions and causes of mortality among sea turtles stranded on the coasts of the Canary Islands. This paper describes morphologic and toxicological findings in a case of pansteatitis in a stranded loggerhead sea turtle *Caretta caretta*.

MATERIALS AND METHODS

A subadult female loggerhead sea turtle was found in March 2009 floating off the coast of Gran Canaria, Canary Islands. The turtle weighed 19.2 kg and had a curved carapace of length and width 55.7 and 57.3 cm, respectively, and a straight carapace length of 54 cm and width of 52.1 cm. The turtle was lethargic, weak, and non-responsive to external stimuli, and was floating in a non-horizontal position, with a lifting of the cranial part of the carapace. The animal died before a health evaluation and medical management could be done at the Tafira Wildlife Rehabilitation Center (TWRC), and the carcass was submitted to the Veterinary Faculty, ULPGC, for post mortem examination.

Necropsy was carried out using the procedures previously described (Orós & Torrent 2001, Flint et al. 2009). Macroscopic lesions were recorded and tissue samples from all major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm for light microscopy and stained with hematoxylin and eosin (HE). Periodic acid-Schiff (PAS) and Ziehl-Neelsen (ZN) stains were used to stain selected sections.

Celomic fat was collected during necropsy, wrapped in aluminium foil and stored at –20°C until analysis. Polychlorinated biphenyls (IUPAC nos. 28, 31, 52, 101, 138, 153, 180, and 209) and DDT and its metabolites (OC-DDTs) (*o,p'*-DDT, *o,p'*-DDE, and *o,p'*-DDD) were analyzed according to the method described by Tanabe et al. (1994). The validity of analytical methods was confirmed with Standard Reference Materials (CARP-2: ground whole carp *Cyprinus carpio*) obtained from the National Research Council of Canada (Table 1).

Aliquots (4 to 7 g) of the homogenized samples were ground with anhydrous sodium sulphate in a mortar, and extracted using Soxhlet apparatus for 6 h with 300 ml of diethyl ether:hexane (3:1) solvent mixture. Extracts were concentrated in volume to 10 ml in Kuderna-Danish and the aliquots (2 ml) were transferred to a glass column packed with 20 g of Florisil and dried by passing through nitrogen gas. Organochlorines adsorbed on Florisil were eluted with 150 ml of 20% hexane-washed water in acetonitrile and transferred to a separatory funnel containing 600 ml of hexane-washed water and 100 ml of hexane. After partitioning, the hexane layer was concentrated, cleaned up with sulphuric acid, and passed through a 12 g Florisil packed glass column for separation.

Table 1. Precision and accuracy of analytical methods obtained using a certified ground whole carp *Cyprinus carpio* (CARP-2). Concentrations are given in ng g^{-1} wet weight. Number of replicates: 3

	CARP-2	
	Certified	Found
PCB 28	34 ± 7.2	31.5 ± 2.6
PCB 52	138 ± 43	120.1 ± 11.1
PCB 101	145 ± 48	148.7 ± 18.3
PCB 138	103 ± 30	99.8 ± 16.8
PCB 153	105 ± 22	115.2 ± 14.8
PCB 180	53.3 ± 13.0	58.7 ± 9.5
PCB 209	4.6 ± 2.0	4.8 ± 3.8
o,p'-DDD	21.8 ± 0.7	18.6 ± 2.9
o,p'-DDE	2.9 ± 0.5	2.6 ± 3.4

Final determination of PCBs and OC-DDTs was carried out using a Varian 3600 gas chromatograph fitted with an electron capture detector (GC-ECD). All analyses used a fused-silica capillary column Supelco (length 30 m, inside diameter 0.53 mm and film thickness 0.50 µm). The column oven was programmed from 60 to 160°C, held for 10 min, and then increased to 260°C at a rate of 2°C min^{-1} and held for 20 min. Injector and detector temperatures were set at 260 and 280°C respectively. Nitrogen was used as carrier gas with 63.3 ml min^{-1}. The internal standard used for quantification of OC-DDTs and PCB congeners was tetrachloro-*m*-xylene. Detectable concentration range for the contaminants included in this study was from 1 ng g^{-1} (instrumental quantification limit) to 50 000 ng g^{-1} (optimum linear limit). Concentrations of contaminants (means of 3 measurements) are presented as ng g^{-1} on a wet weight basis.

Samples from liver, celomic wall and celomic fat were also taken and cultured on a variety of selective and non-selective media including blood agar (Oxoid), MacConkey agar (Oxoid), Baird Parker agar (Oxoid) for staphylococci, and Sabouraud Dextrose agar (Oxoid) for fungi and yeasts. All cultures were incubated at 25°C aerobically.

RESULTS

At necropsy a generalized subcutaneous edema was observed. Large amount of adipose tissue in the celomic cavity showing very firm, yellow to orange irregular formations was observed (Fig. 1). At the cut surface the fat showed orange to brown irregular nodules and was mildly hyperhemic (Fig. 1). Fat samples did not float in the formalin solution. The liver was cranially displaced and presented a reduced size. Several nematode larvae were observed in the gastric serosa. No gross lesions were visible in other major organs.

Histologically, the celomic fat presented severe extensive degeneration and necrosis. Lesions ranged from the infiltration of necrotic fat by scarce multinucleated giant cells and numerous macrophages containing xylene-insoluble lipopigment inclusions to the presence of several granulomas characterized by an irregular central area consisting of necrotic fat showing variable amounts of lipopigment surrounded by numerous multinucleated giant cells (Fig. 2). In addition, granulomas consisting of varyingly enlarged as well as ruptured adipocytes surrounded by ceroid-containing macrophages were observed. Presence of ceroid pigment in the macrophages and in the areas of necrotic fat was confirmed by staining with ZN (Fig. 2, inset) and PAS stains. No microorganisms or parasites were identified in the lesions of the fat. Several granulomas associated a nematode larvae were observed in the lamina propria, submucosa, lamina muscularis and serosa of the stomach. No histological lesions were observed in other organs. All microbiological cultures were negative.

Fig. 1. *Caretta caretta*. Large amount of adipose tissue showing very firm irregular formations in the left part of the celomic cavity. Note the dark nodule (arrow) in the cut surface. Scale bar = 2.2 cm

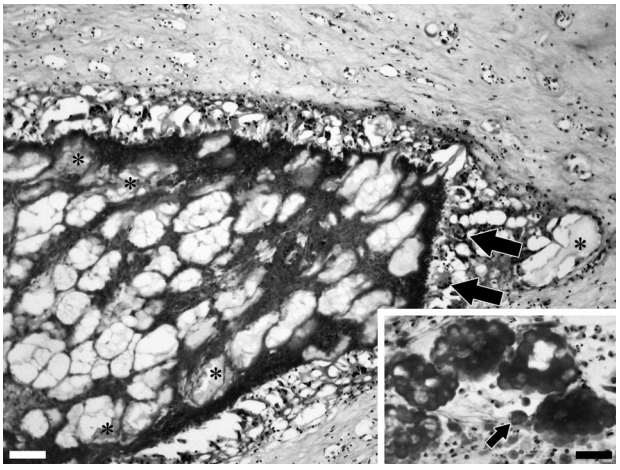

Fig. 2. *Caretta caretta*. Granuloma in the celomic fat characterized by a central area of fat necrosis with variable amounts of lipopigment (*) surrounded by numerous multinucleated giant cells (arrows). HE stain. Scale bar = 80 µm. Inset: Acid-fast staining of the ceroid pigment in areas of necrotic fat and within macrophages (arrow). ZN stain. Scale bar = 55 µm

Concentrations of PCBs and OC-DDTs in the celomic fat of this turtle are presented in Table 2.

DISCUSSION

Histological examination of the celomic adipose tissue of this turtle confirmed the diagnosis of pansteatitis. The presence of ceroid-laden macrophages and characteristic multinucleated giant cells surrounding degenerating and ruptured adipocytes as well as the associated granuloma formation are typical findings in all reptile species suffering from

Table 2. *Caretta caretta*. Concentration of PCBs and OC-DDTs (ng g^{-1} wet wt) in the celomic fat of the loggerhead turtle suffering from pansteatitis. BDL: below detection limit (1 ng g^{-1})

	Contaminant	Concentration
PCB	28,31	5.1
	52	4.8
	101	7.8
	138	3170
	153	2830
	180	980
	209	1190
o,p'-DDT		6.3
o,p'-DDD		5.9
o,p'-DDE		BDL

pansteatitis (Larsen et al. 1983, Huchzermeyer 2003, Manawatthana & Kasorndorkbua 2005, Bouwman et al. 2009). In addition, similar lesions have been reported in several fish species (Begg et al. 2000, Goodwin 2006, Roberts & Agius 2008, Huchzermeyer et al. 2011). This is the first report of pansteatitis in a wild sea turtle.

In a study on the lipid properties of pansteatitis-affected wild Nile crocodiles the authors concluded that the hard texture of celomic fat with steatitis was not caused by the fatty acid composition, but could be ascribed to the mononuclear cellular infiltrate associated with the inflammatory process (Osthoff et al. 2010). Buoyancy problems are common in sea turtles and are not always associated with pulmonary lesions (Orós et al. 2005). In the absence of pulmonary and intestinal lesions the large amount of very hard fat irregularly distributed mainly in the caudal part of the celomic cavity observed in this turtle could explain the abnormal floatation.

Pansteatitis has been defined as a nutritional disorder characterized by inflammation of adipose tissue and deposition of ceroid pigment in fat cells as a result of vitamin E deficiency (Osthoff et al. 2010). Fytianou et al. (2006) provided experimental evidence linking the disease to the consumption of high levels of unsaturated fatty acids or oxidized fats that deplete the vitamin E.

Pansteatitis in reptiles has been previously associated with an exclusive fish diet in captive alligators (Larsen et al. 1983), consumption of large numbers of dead and rancid fish in farmed crocodiles (Huchzermeyer 2003), and exclusive frozen fish diet for more than 20 yr without proper vitamin supplementation in captive olive ridley sea turtles (Manawatthana & Kasorndorkbua 2005). In 2008 an episode of crocodile pansteatitis occurred in the Kruger National Park (KNP), South Africa. The primary cause of this outbreak remains unknown, but it is speculated that the deaths were triggered by a combination of environmental and dietary factors (Bouwman et al. 2009). Sediment from the rivers in the KNP contained traces of dioxin-like substances, some pesticides (DDT and HCH), polycyclic aromatic hydrocarbons, and polybrominated flame-retardants. However, the levels of organochlorine contaminants found in the fat of dead crocodiles were not particularly high, and none of their methylsulfone metabolites were detected (Bouwman et al. 2009). In addition, Begg et al. (2000) reported steatitis in 2 species of wild marine fish, with suspicion of a pollution-related etiology, and changes in antioxidant parameters in Nile tilapia *Oreochromis niloticus* have been linked to the effects of oxidative stress from polluted water (Bainy et al. 1996).

The high levels of PCB 138, 153, 180 209 found in the celomic fat of this loggerhead turtle are very remarkable. In a previous study on PCBs in 30 loggerhead turtles stranded in the Canary Islands, the mean concentrations in fat for PCB 138, 153, 180 209 were 83, 133, 217, and 15 ng g^{-1}, respectively, and the highest individual concentrations in fat for PCB 138, 153, 180 209 were 370.5, 2600, 5006, and 218 ng g^{-1}, respectively (Orós et al. 2009). Authors concluded that tissues from those turtles contained higher levels of PCBs than those reported in turtles from other geographical regions. The levels of PCB 138, 153 209 in the turtle from the current study are the highest PCB values reported for a sea turtle (D'Ilio et al. 2011). In addition, these values were similar to those reported for bottlenose dolphins *Tursiops truncatus* stranded in the Canary Islands (Carballo et al. 2008), suggesting that this turtle was feeding on fish.

The concentrations of *o,p'*-DDT, *o,p'*-DDE, and *o,p'*-DDD in the fat of this loggerhead turtle were lower than those reported previously in a survey on 23 loggerhead turtles stranded in the Canary Islands (Monagas et al. 2008). However, because of the specific research interest of our department in the *o,p'*-DDT as estrogenic environmental contaminant compared to synthetic estrogens, *p,p'*-isomers were not measured. And, according to the bibliography, *p,p'*-DDE is the pesticide present in the greatest concentrations in sea turtles due to its highly persistent nature (D'Ilio et al. 2011).

Sea turtle blood is also a suitable alternative to fatty tissues for measuring organochlorine contaminants because blood concentrations reasonably represent those observed in the paired fat samples (Keller et al. 2004a). Paired blood plasma samples would have been informative to clarify contaminant distribution, as deposition to and remobilization from fat stores could have been heavily impacted by pansteatitis.

PCBs are organic pollutants that persist and bioaccumulate in the environment. Studies on the effects of these contaminants on diseases and lesions of sea turtles are scarce (Keller et al. 2004b, Swarthout et al. 2010). Previous studies have shown that they increase oxidative stress in many model systems including mammals (Wells et al. 1997), birds (Jin et al. 2001) and fish (Otto & Moon 1996). In fact, changes in the oxidative status due to the exposure to contaminants, such as certain PCB congeners (e.g. non-ortho-substituted and mono-ortho-substituted congeners), leads to generation of reactive oxygen

species and this has been linked with the development of lipid peroxidation and immunotoxicity in aquatic organisms (Coteur et al. 2001, Shimizu et al. 2007). There is growing evidence that both mechanisms can be involved in the development of steatitis (Begg et al. 2000, Fromenty et al. 2004). Although a nutritional cause (i.e. consumption of an abnormal quantity of oily fish with a high concentration of unsaturated fatty acids) for the pansteatitis observed in this wild loggerhead turtle cannot be ruled out, the high levels of PCBs detected in the celomic fat could have induced lipid peroxidation in adipocytes of this animal, resulting in cell damage, deposition of ceroid pigment and inflammatory response. The cause of the high levels of PCBs in this turtle could be a diet of higher trophic level, lipid-rich prey. However, the major diet components for loggerhead turtles in this geographical area are epipelagic salps, gastropods, pelagic coelenterates (primarily siphonophores and to a lesser extent Scyphomedusae and Hydromedusae), and barnacles (Van Nierop & den Hartog 1984). Because loggerhead turtles are primarily carnivorous opportunistic feeders, feeding of some loggerheads primarily on fisheries bycatch could not be discarded.

Because data of the effects of PCBs on diseases and lesions of sea turtles are scarce, this report, although based on a single individual, provides an important addition to the association between PCBs and disease in sea turtles, and suggests the need to investigate the role of these contaminants in the pathogenesis of pansteatitis in vertebrates.

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