Journal of Wildlife Diseases, 46(1), 2010, pp. 111–117

COMPARISON OF MERCURY BURDENS IN CHRONICALLY DEBILITATED AND HEALTHY LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*)

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ABSTRACT: An increase in the incidence of debilitated loggerhead sea turtle (*Caretta caretta*) strandings in the southeastern United States has been observed in recent years. These turtles are characterized by emaciation and heavy burdens of external and internal parasites, and bacterial infections, but the underlying cause of their condition is unknown. To investigate further the causes of these strandings, a health assessment was performed on stranded, debilitated loggerhead turtles, and contaminant concentrations in various tissues were compared to those from healthy turtles. This portion of the study investigated the potential role of mercury (Hg) toxicity in the debilitated condition described above. Hematocrit, total protein, albumin, globulin, glucose, calcium, lymphocyte counts, heterophil:lymphocyte ratios, aspartate aminotransferase, uric acid, sodium, and chloride were altered in debilitated loggerheads relative to healthy animals. However, none of the aforementioned health indicators correlated with Hg concentrations in either red blood cells (RBCs) or plasma. The Hg concentration in RBCs was 129±72 (mean±standard deviation) times higher than in plasma, causing a significant dilution of Hg in whole blood due to extreme anemia. Mercury concentrations in RBCs (73.7±21.2 ng/g) and scutes (455±57 ng/g) from debilitated turtles were similar to or lower than those reported for healthy animals, indicating no elevation in Hg exposure before and during the progression of this condition. These findings suggest that Hg toxicity does not play a role in the debilitated loggerhead condition observed in the southeastern United States.

Key words: Blood, disease, emaciation, keratin, loggerhead sea turtle, mercury, plasma, red blood cell.

INTRODUCTION

From 1992 to 2003, there was a perceived increase of sea turtle strandings (dead or moribund) in the southeastern United States in which turtles were classified as "debilitated." In 2003, this was punctuated by a notably sharp increase in the numbers of debilitated loggerhead sea turtle (*Caretta caretta*) strandings and the percentage of the total strandings that were classified as debilitated. From 1992 to 2002, South Carolina reported an average of five debilitated loggerheads per year, representing 5% of the total strandings, while in 2003 this increased to 37 strandings, comprising 28% of the total. A similar trend was observed in Georgia, with an increase from the 10-yr average of three strandings (1% of total) to 29 strandings (11% of total) in 2003. A debilitated turtle is defined as emaciated, with small barnacles covering the skin, suggesting a recent, sustained period of reduced activity. The flippers may also have lesions or may be necrotic. While heavy epibiota can be a normal finding on the carapace and plastron of healthy sea turtles, the skin is generally free of these commensals. A comparison between a healthy loggerhead sea turtle and individuals characterized as

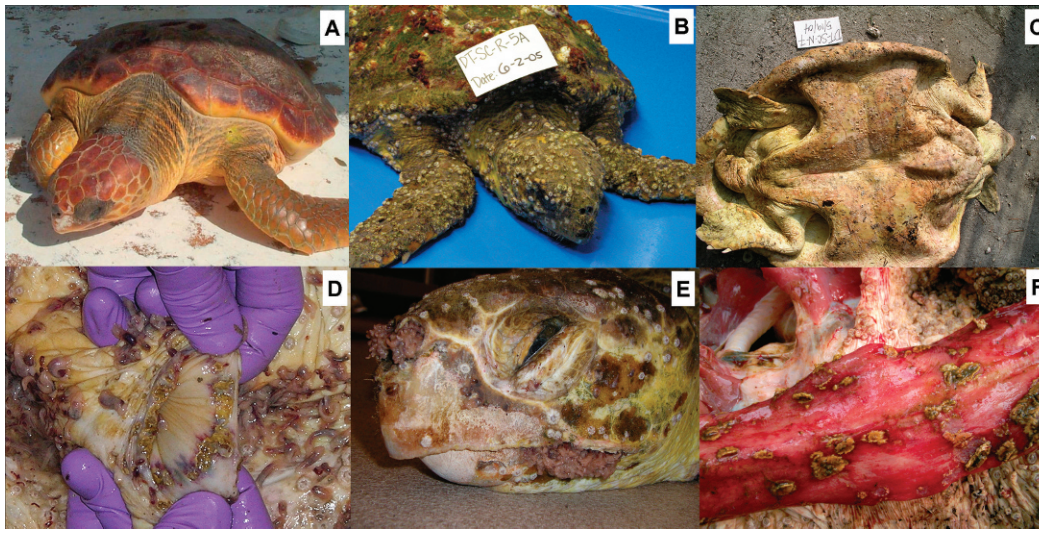

FIGURE 1. (A) Dorsal anterior view of a typical, healthy loggerhead sea turtle. (B) Dorsal anterior view of a typical debilitated turtle exhibiting heavy barnacle and other epibiotic growth on skin and carapace, emaciated neck, and sunken eyes. (C) Ventral view showing concave and sunken plastron. (D) Cloaca and surrounding skin severely infested by leeches and leech eggs. (E) Nares and mouth occluded with leeches and leech eggs and bryozoan growth on side of head. (F) Caseous plaques inside the intestinal wall.

debilitated is presented in Fig. 1A–C. Loggerhead sea turtle were the predominant species affected, and strandings were most heavily concentrated in the spring and summer (April through July) in all states but Florida, where strandings occurred all year. There also appeared to be areas of higher stranding incidence in southern North Carolina, northern South Carolina, and near Cape Canaveral, Florida (Norton et al., 2005).

Preliminary health assessment and necropsy data from debilitated turtles sampled along the southeastern coast of the United States indicated that turtles were being affected by a wide range of secondary bacterial and parasitic infections (Fig. 1D–F), but the primary cause of their condition could not be determined. Seven debilitated turtles, sampled as part of a previous study, showed significantly higher blood concentrations of polychlorinated biphenyls and organochlorine pesticides compared to 47 apparently healthy turtles (Keller et al., 2006). In a separate study, mercury (Hg) concentrations in blood and scutes were two- to three-times higher in dead stranded turtles compared to live, apparently healthy turtles (Day, 2003). These higher contaminant burdens in stranded and debilitated turtles prompted the question of whether immunotoxicity could be responsible for the onset of secondary infections that led to a decline in health. Significant negative correlations have been found between blood Hg concentrations and lymphocyte cell count and B-cell proliferation rates in loggerhead sea turtles (Day et al., 2007). In vitro exposure of B-cells to methylmercury (MeHg) in the same study also showed significant suppression of B-cell proliferation at environmentally relevant concentrations. However, because most of the individuals in the previous study appeared to be in good health, it was unclear whether Hg at these levels actually contributed to higher rates of disease. In this study, we compare Hg burdens in debilitated loggerhead sea turtles to those in healthy turtles and examine the relationships between Hg concentrations and clinical parameters.

MATERIALS AND METHODS

Debilitated turtles were defined as emaciated with small barnacles covering the skin (Fig. 1B, C). During 2004–2005, samples of blood and scutes were collected from live, moribund, and dead debilitated turtles that were stranded from North Carolina to Florida. Scute samples from dead turtles were only collected from freshly dead individuals, and no blood was collected. An extensive protocol was developed to standardize the necropsies and the collection and processing of tissues for diagnostic and analytical evaluation, and to prevent contamination or compromise of the samples. Approximately 5 ml of blood was collected from the dorsocervical sinus of live and moribund turtles ($n=16$; Owens and Ruiz, 1980), centrifuged for 5 min to separate red blood cells (RBCs) and plasma, and frozen upright at −20 C in the original blood-collection tube. Prior to analysis, samples were thawed, plasma and RBCs were removed, each fraction was vortexed, and 1–3 g was weighed for analysis. Keratin was collected from the posterior marginal scutes, according to a previously described methodology (Day et al., 2005), and stored at −80 C until analysis. Scute samples were collected from both live and dead strandings ($n=44$).

Total Hg concentration (based on wet mass) in blood and scutes was determined using isotope dilution cold vapor inductively coupled plasma mass spectrometry at the National Institute of Standards and Technology (Hollings Marine Laboratory, Charleston, South Carolina, USA) using methods described in detail elsewhere (Christopher et al., 2001). Samples were prepared in quartz vessels using microwave-assisted acid digestion (Perkin-Elmer Multiwave, Shelton, Connecticut, USA) and analyzed on a Plasma Quad 3 ICPMS (VG Elemental, Winsford, UK). National Institute of Standards and Technology standard reference materials were run concurrently within each analytical batch (~10 unknown samples) for method validation. The SRM 2976 mussel tissue (trace elements and methylmercury), freeze-dried (61.0±3.6 ng/g, dry mass basis), was run with scute samples, and SRM 966 toxic metals in bovine blood (29.8±1.6 ng/g, wet mass basis) was run with blood samples. Measured values of SRM 2976 were 61.2±2.1 ng/g (mean±1SD, n=7), and measured values of SRM 966 were 29.6±1.9 ng/g (mean±1SD, $n=3$). Plasma chemistries (total protein, albumin, globulin, glucose, calcium, phosphorus, potassium, sodium, chloride, aspartate aminotransferase, creatine phosphokinase, urea nitrogen, uric acid,) and blood cell counts (hematocrit, lymphocyte counts, heterophil:lymphocyte ratios) were performed by Antech Diagnostics (Memphis, Tennesee, USA). Complementary contaminant analysis, immunology, histology, and parasitology were also performed, but are not addressed here. An extensive set of internal tissues (liver, kidney, brain, etc.) was also collected, but based on the results presented here, these tissues were not further analyzed for Hg.

Mercury concentrations in debilitated loggerheads were compared to a healthy reference group, reported in the literature from the same region and time period, for both scutes (Day et al., 2005) and blood (Day et al., 2005; 2007). As the aforementioned studies measured whole blood, and the current study measured RBCs and plasma separately, a conversion was performed that allowed a tissue-matched comparison. This conversion used the relative concentrations of Hg in RBCs and plasma reported in the current study and the hematocrit count measured for each blood sample (measured in both studies) to reconstruct whole blood or RBC concentrations. Standard, one-way analysis of variance (ANOVA) was used to test for differences among means, and all assumptions of ANOVA were confirmed prior to analysis. All statistics were performed on SAS JMP Ver 4.02 (SAS, Cary, North Carolina, USA).

RESULTS

No correlations were found between Hg concentrations and any of the measured clinical plasma chemistries (total protein, albumin, globulin, glucose, calcium, phosphorus, potassium, sodium, chloride, aspartate aminotransferase, creatine phosphokinase, urea nitrogen, uric acid), lymphocyte counts, or heterophil:lymphocyte ratios. The mean hematocrit in the healthy reference population of loggerheads was 36%, while in debilitated turtles it was 8%. The Hg concentrations in RBCs ($n=16$) and plasma ($n=16$) were 73.7±33.8 ng/g and 0.6±0.2 ng/g, respectively (mean±standard deviation) (Fig. 2). The mean ratio of Hg concentrations in these two fractions (129±72), and the hematocrit, were used to convert whole blood Hg concentrations from healthy animals into RBC Hg concentration for comparison to debilitated turtles. There

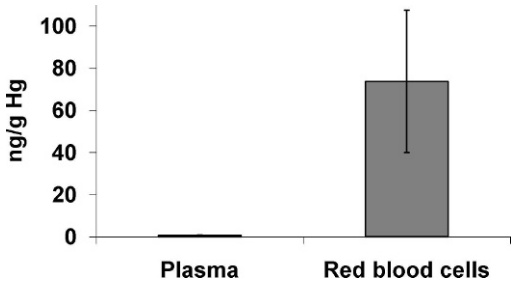

FIGURE 2. Hg concentrations (mean±standard deviation) in the red blood cell (73.7±33.8 ng/g) and plasma (0.6±0.2 ng/g) fractions of whole blood in 16 debilitated loggerhead sea turtles. The ratio of Hg in RBCs/plasma was 129±72.

was no difference (ANOVA, $P=0.64$) between red blood cell Hg concentrations in healthy, live-captured loggerhead sea turtles (85.7±14.5 ng/g; $n=34$; Day et al., 2005) and chronically ill debilitated loggerhead sea turtles (73.7±21.2, $n=16$). Whole blood Hg concentrations were significantly lower ($P=0.01$) in debilitated turtles (6.4±7.3 ng/g, $n=13$) than in healthy turtles (29.5±4.5 ng/g; $n=100$; Day et al., 2005; 2007) due to their extremely anemic condition (Fig. 3). There was also no difference (ANOVA, $P=0.95$) between scute Hg concentrations in healthy, live-captured loggerhead sea turtles (461±64 ng/g; $n=34$; Day et al., 2005) and chronically ill, debilitated loggerhead sea turtles (455±57 ng/g, $n=44$) (Fig. 4).

DISCUSSION

The loggerhead turtles sampled as part of this study were in an extremely advanced stage of debilitation. It is impossible to ascertain how long, prior to stranding, the onset of this condition began, but given the degree of emaciation and the potentially slow metabolism of sea turtles, it is reasonable that these animals may have been in this wasting condition for months. In addition to the gross observations noted during necropsy, the clinical diagnostic parameters in these animals showed that virtually all of the parameters measured were significantly altered from normal, healthy values (Norton, unpubl. data). However, no correlations were found between Hg concentrations and any of the measured clinical plasma chemistries or blood cell counts.

The primary goal of the current study was to document the Hg burdens in debilitated loggerheads and compare these concentrations to those observed in healthy loggerheads from the same region. This was part of a larger effort to characterize their condition in terms of their clinical, immune, and contaminant status. Blood was one tissue selected for initial Hg analysis for several reasons. First, it is well established that blood is the primary transport mechanism for moving Hg throughout the body, and delivering it to target organs, where toxicologic effects can occur. It has been previously demonstrated in loggerhead sea turtles that Hg levels in blood are representative of the Hg burdens in other organs (Day et al., 2005). Second, this tissue is also typically used for clinical diagnostics and immune parameters, and correlations have been observed between blood Hg concentrations and several of these health indicators (Day et al., 2007).

It was known prior to beginning this study that turtles in the observed debilitated state are often extremely anemic, and hematocrit values can vary dramatically. Positive correlations between Hg concentrations in whole blood and hematocrit have been observed in sea turtle health assessments targeting apparently healthy populations, where hematocrit values are not highly variable (Day et al., 2007). This is not of toxicologic significance, but instead is an artifact of the higher affinity of MeHg to RBCs than to plasma (Suzuki et al., 1971; Ansari et al., 1973; Kershaw et al., 1980; Magos, 1987; USEPA, 1997). The mean hematocrit in the debilitated turtles was more than four times lower than the healthy reference population of loggerheads, causing a significant dilution of the more Hg-rich RBC fraction with plasma. Therefore, it

was particularly important to measure Hg in the RBC and plasma fractions of the whole blood separately, as previously suggested in the literature (Kershaw et al., 1980; Day et al., 2007).

The mean ratio of Hg concentrations in the RBC and plasma fractions (129±72) and the hematocrit were used to convert whole blood Hg concentrations from healthy animals into RBC Hg concentration for comparison to debilitated turtles. It is interesting to note that the variability measured in this ratio is high. The Hg levels in these two compartments attain a relatively rapid equilibrium (Rabenstein et al., 1982; 1986) and, under normal conditions, one would expect the binding affinity of Hg in these two fractions to be fairly consistent and the concentrations to be highly correlated. It is possible that the high variability in the RBC/plasma Hg concentration reported here is due to significant and highly variable changes in the cellular or plasma fractions, or both, of the blood, such as concentration of total solids, hemolysis, bile intrusion, or dehydration status. Due to the very low Hg concentration in plasma, the uncertainty associated with this conversion term is insignificant for the results presented here.

Using whole blood would have resulted in the measurement of significantly lower Hg levels in debilitated turtles compared to healthy turtles due to their anemic condition (Fig. 3). However, comparing RBC Hg concentrations in debilitated and healthy turtles shows that no difference in Hg concentrations exists (Fig. 3). While blood is a reliable measure of Hg exposure, this tissue is dynamic and can be influenced by recent changes in exposure or physiology. Because the onset of the debilitated condition of these animals could have begun months prior to sampling, it is possible that cessation of feeding could create an artificially low blood Hg level that is not representative of the burden during the beginning of their health decline. However, the scute Hg

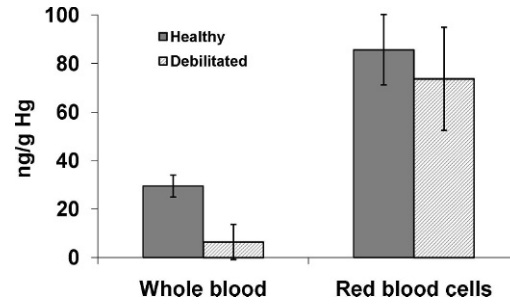

FIGURE 3. There was no difference (ANOVA, $P=0.64$) between red blood cell Hg concentrations in healthy, live-captured loggerhead sea turtles (85.7±14.5 ng/g, $n=34$) (Day et al., 2005) and chronically ill, debilitated loggerhead sea turtles (73.7±21.2, $n=16$). Whole blood Hg concentrations were significantly lower ($P=0.01$) in debilitated turtles (6.4±7.3 ng/g, $n=13$) than in healthy turtles (29.5±4.5 ng/g, $n=100$) (Day et al., 2005; 2007) due to their extremely anemic condition. Bars=mean±1 standard error.

concentrations are not sensitive to these transient changes because they represent a longer period of exposure than does the blood. Like mammalian hair or bird feathers, the outer corneous layer of turtle scutes are made of compacted keratinocytes that become metabolically inactive and are eventually sloughed off (Alibardi, 2005). Therefore, the Hg that is strongly bound to keratin proteins in scutes (Crewther et al., 1965) is unavailable for remobilization. The scute Hg concentrations in debilitated and healthy loggerheads were remarkably similar (Fig. 4), indicating both groups experienced similar levels of Hg exposure prior to their terminal condition.

The similar Hg burdens in apparently healthy, free-ranging animals captured in the wild, compared to debilitated turtles, suggest that Hg does not play a role in the presentation of the debilitated condition in loggerheads in the southeastern United States. Based on these findings, additional analyses of Hg in brain, liver, kidney, or other organ systems were not performed. Results of the studies on organic contaminants, parasitology, histology, immunology, and clinical pathol-

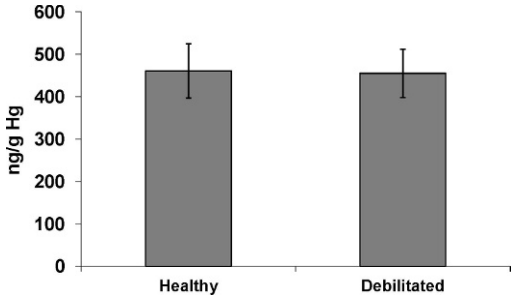

FIGURE 4. There was no difference (ANOVA, $P=0.95$) between scute Hg concentrations in healthy, live-captured loggerhead sea turtles (461±64 ng/g; $n=34$; Day et al., 2005) and chronically ill, debilitated loggerhead sea turtles (455±57 ng/g, $n=44$). Bars=mean±1 standard error.

ogy are pending. Due to the abundance of secondary infections and general deterioration in health, it is very difficult to identify the etiology of the debilitated condition observed in this study. It is unlikely that only a single, underlying etiology exists, and this condition represents the final stages of deterioration in health that could result from a variety of sources.

ACKNOWLEDGMENTS

The authors would like to thank the National Oceanic and Atmospheric Administration's Protected Resource Division for partial funding of this work. The authors would also like to thank all of the state stranding personnel and volunteers in Florida, Georgia, South Carolina, and North Carolina, the Mote Marine Laboratory, the South Carolina Aquarium, and the St. Catherine's Island Wildlife Center. Certain commercial equipment or instruments are identified in the article to adequately specify the experimental procedures; such identification does not imply recommendations or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment or instruments are the best available for the purpose.

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Received for publication 10 December 2008.