Vol. 95: 163–166, 2011
doi: 10.3354/dao02351

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published June 16

Salt gland adenitis as only cause of stranding of loggerhead sea turtles *Caretta caretta*

J. Orós[1,*], M. Camacho[1], P. Calabuig[2], A. Arencibia[1]

[1]Department of Morphology, Veterinary Faculty, University of Las Palmas de Gran Canaria (ULPGC), Arucas (Las Palmas) 35416, Spain

[2]Tafira Wildlife Rehabilitation Center, Tafira Baja, Las Palmas de Gran Canaria 35017, Spain

ABSTRACT: The present study describes pathological and microbiological findings in 9 stranded loggerhead sea turtles *Caretta caretta*, whose only observed lesion was bilateral purulent salt gland adenitis. Histological lesions ranged from the presence of abundant eosinophilic material associated with bacterial colonies in the lumen of the central ducts of the glandular lobules to the destruction of the glandular tissue and presence of abundant eosinophilic material composed of heterophils and cell debris, lined by multinucleated giant cells. *Aeromonas hydrophila*, *Staphylococcus* sp., and *Vibrio alginolyticus* were the bacteria most frequently isolated. Plasma concentrations of sodium and chloride and plasma osmolality from 2 turtles suffering from salt gland adenitis were, respectively 45.7, 69.2, and 45.7% higher than the mean value for healthy turtles. These cases suggest that failure to maintain homeostasis due to severe lesions in the salt glands can cause stranding and/or death of loggerhead sea turtles.

KEY WORDS: Sea turtle · Salt gland · *Caretta caretta* · Loggerhead

INTRODUCTION

All species of sea turtles are included in the IUCN's Red List of Threatened Species (www.iucnredlist.org). In recent years, increased efforts have been devoted to the conservation of sea turtles, including medical management and pathological studies on stranded animals (Glazebrook & Campbell 1990b, Gordon et al. 1993, Work et al. 2004, Orós et al. 2005).

Sea turtles live in an osmotically challenging environment where the concentration of salt is approximately 3 times greater than that of their internal fluids (Reina et al. 2002). The paired lachrymal salt glands allow sea turtles to maintain ionic homeostasis in a hypersaline environment by secreting a concentrated sodium chloride solution in response to increased plasma sodium (Reina 2000). There are very few descriptions of salt gland diseases in wild sea turtles in the literature (Orós et al. 2005, Flint et al. 2009). No confirmed reports of salt gland adenitis as the only cause of stranding of sea turtles could be found in the literature.

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 the morphologic and clinical pathology of salt gland adenitis as a cause of stranding in loggerhead turtles.

MATERIALS AND METHODS

The 9 loggerhead turtles studied were stranded on the coasts of the Canary Islands between January 2002 and November 2009. Of these, 4 had been previously presented to the Tafira Wildlife Rehabilitation Center (TWRC) for health evaluation, medical management, and possible rehabilitation. The minimum and maximum times in rehabilitation were, respectively, 2 and

*Email: joros@dmor.ulpgc.es

12 d, and subsequently all turtles were submitted for necropsy to the Veterinary Faculty at ULPGC.

In addition, 25 loggerhead turtles evaluated as clinically healthy and maintained in outdoor facilities with sea water belonging to the TWRC throughout 2008 and 2009 were used for establishing reference values for plasma concentrations of sodium and chloride and plasma osmolality. Clinical evaluation included physical examination, evaluation of swimming activity, core body temperature (measured from the cloaca), food ingestion, weight to straight carapace length ratio, hydration, and biochemical and hematological pattern.

One ml of blood was collected from the cervical sinus of 25 healthy and 2 diseased turtles (Owens & Ruiz 1980) into 30 IU of lithium heparin (Teramo Europe N. V.) with a 2 ml syringe and a 2.5 × 0.6 mm needle. Blood was placed in plastic tubes (1.5 ml capacity; Eppendorf Ibérica), preserved at 4°C and, about 30 min after blood collection, centrifuged for 15 min at 10 000 × *g* (Mixtasel centrifuge; Selecta); the plasma was then immediately separated using a Pasteur pipette. Plasma was stored for up to 10 d at –20°C while waiting for analysis.

Plasma concentrations of sodium and chloride were measured using an automated chemistry analyzer fitted with an ion selective electrode system (Olympus AU460), and ISE reagents and standards (Olympus) according to the manufacturer's instructions. Plasma osmolality was measured using a vapor pressure osmometer (Wescor Vapro 5520).

Necropsies were carried out using procedures previously described by Wolke & George (1981) and 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). Special stains performed on all the cases included Ziehl Neelsen (ZN) for acid-fast organisms, and periodic acid-Schiff (PAS) and Grocott's methenamine silver nitrate (GMS) for fungi.

Samples were also taken from gross lesions and cultured on a variety of selective and non-selective media (Oxoid), including blood agar, MacConkey agar, Baird Parker agar for staphylococci, and Sabouraud Dextrose agar for fungi and yeasts. All cultures were incubated aerobically at 25°C. Once a pure growth was obtained, bacteria were identified based on the biochemical profile (API 20 E, API 20 NE, and API 20 Staph, bioMérieux).

RESULTS

Of the stranded turtles, 7 were female and 2 were male. The means ± SD of the straight carapace length and weight of the turtles were 35.3 ± 4.8 cm (range: 26.8 to 47.2 cm) and 11.1 ± 1.5 kg (range: 3.6 to 24.2 kg), respectively. On the basis of straight carapace length and sexual maturity (estimated from the appearance of their gonads), all turtles were identified as juvenile or subadult specimens.

Sea turtles submitted to the TWRC for health evaluation showed lethargy and anorexia. At necropsy the 9 turtles showed severe bilateral purulent salt gland adenitis. Notable amounts of yellow caseous necrotic debris were detected throughout the parenchyma of the salt glands, occupying areas of up to 2.5 cm in diameter (Fig. 1). No gross lesions were visible in other organs of these turtles.

Histologically, a severe heterophilic salt gland adenitis was diagnosed in the 9 turtles. Lesions ranged from the presence of abundant eosinophilic material associated with bacterial colonies in the lumen of the central ducts of the glandular lobules to the destruction of the glandular tissue and presence of abundant eosinophilic material composed of heterophils and cell debris, lined by multinucleated giant cells. Multinucle-

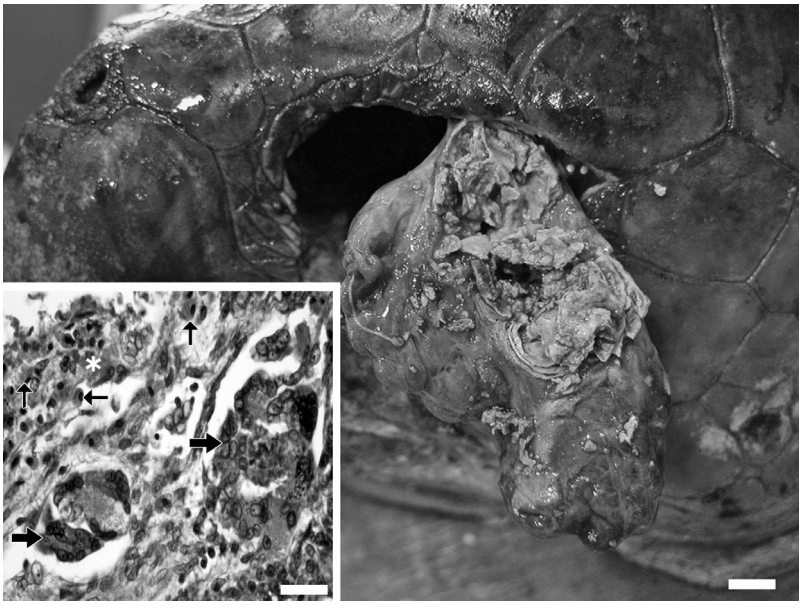

Fig. 1. *Caretta caretta*. Severe purulent salt gland adenitis. Scale bar = 1 cm. Inset: multinucleated giant cells (thick arrows) around an inflammatory focus in a glandular lobule. Note also the heterophils (thin arrows), some of which are degranulated (white star). HE stain. Scale bar = 25 µm

ated giant cells were detected around the inflammatory foci in all cases (Fig. 1, inset). Other lesions included interstitial edema and presence of numerous heterophils in the interstitial tissue. No ZN-, PAS- or GMS-positive microorganisms were detected. No histological lesions were detected in other organs.

The bacteria isolated from the lesions of the salt glands of the stranded turtles were: *Aeromonas hydrophila* (n = 3), *Staphylococcus* sp. (n = 3), *Vibrio alginolyticus* (n = 3), *Aerococcus viridans* (n = 1), and *Citrobacter* sp. (n = 1). A pure culture was obtained from 7 turtles, whereas a mixed culture was obtained from 2 turtles (*A. viridans* with *V. alginolyticus*, and *A. hydrophila* with *Citrobacter* sp.). No fungi or yeasts were isolated from the salt glands.

The means ± SD of the straight carapace length and weight of the 25 healthy turtles were 32.3 ± 8.9 cm (range: 18 to 46.5 cm) and 6.18 ± 4.1 kg (range: 1 to 15.3 kg), respectively.

The mean ± SD of plasma concentrations of sodium and chloride (mmol l^{-1}), respectively, was 218.5 ± 2.5 and 187 ± 5 for 2 diseased turtles and 149.9 ± 2.5 and 110.5 ± 4.2 for 25 healthy turtles. The mean ± SD of plasma osmolality (mOsm kg^{-1}) was 527.5 ± 21.5 for 2 diseased and 361.8 ± 5.9 for 25 healthy turtles, respectively.

DISCUSSION

The severity of the salt gland lesions and the absence of other gross and histological lesions highlight the significance of salt gland adenitis in the differential diagnosis of stranding in marine turtles.

Because the kidney of the sea turtles cannot produce hypertonic urine, excess salt entering the body by ingestion of food or sea water is largely excreted by paired salt glands (Reina et al. 2002). The salt glands are the largest glands in the head of sea turtles and are found dorsal, medial, and posterior to the eye (Wyneken 2001). Salt glands are composed of specialized, secretory cells that concentrate sodium and chloride from the blood to the lumen of secretory tubules through an energy-dependent process (Abel & Ellis 1966). Sea turtle salt glands secrete a solution composed almost entirely of sodium chloride at approximately 1500 to 1800 mOsm l^{-1} (Marshall & Cooper 1988, Nicolson & Lutz 1989, Reina & Cooper 2000) in response to increasing plasma sodium concentration, and their activity is regulated by microcirculatory changes in or near the glands (Reina 2000).

Very few descriptions of salt gland diseases in wild sea turtles were found in the literature. Calculi have been described in severely dehydrated marine turtles (Flint et al. 2009). Granulomas associated with spirorchiid eggs, of variable severity, are also common in stromal tissue that surrounds central canals of lobules and may sometimes extend into and disrupt the glands themselves (Flint et al. 2009).

None of the sea turtles included in our study had intravascular adult flukes or spirorchiid eggs in tissues, including the salt glands. In a previous study on 93 sea turtles stranded on the coasts of the Canary Islands, no adult flukes or trematode eggs in tissues were detected. Authors hypothesized that different alimentary habits of those juvenile and subadult specimens could explain the absence of exposure to cercaria-rich intermediate hosts (Orós et al. 2005).

In a previous survey, heterophilic adenitis was observed in the salt glands of 2 sea turtles (Orós et al. 2005). Because these turtles showed other lesions in other major organs, lesions in the salt glands were not established as the only cause of stranding. No microbiological isolation was attempted.

Bacteria isolated from the salt glands of the stranded loggerhead turtles included microorganisms which can be considered to be potentially pathogenic and/or opportunistic bacteria. *Vibrio alginolyticus* is regarded as a normal inhabitant of seawater (Roberts 1978), but this microorganism was also isolated repeatedly from cases of traumatic ulcerative dermatitis, ulcerative stomatitis, obstructive rhinitis, and bronchopneumonia in green turtles *Chelonia mydas* (Glazebrook & Campbell 1990a, Glazebrook et al. 1993). In addition, green turtles in Hawaii with fibropapillomas were found to be bacteremic, with 4 species of *Vibrio* spp. representing a majority of the bacteria isolated (Work et al. 2003). *Aeromonas hydrophila* has long been recognized as an opportunistic pathogen of reptiles (Reichenbach-Klinke & Elkan 1965, Paré et al. 2006), including loggerhead sea turtles (Orós et al. 2005). In a previous survey on stranded sea turtles from the Canary Islands, *Staphylococcus* was the genus most frequently isolated (22.58%) from lesions in other major organs (Orós et al. 2005). *Aerococcus viridans* caused fibrinonecrotic inflammation in an esophageal diverticulum in a juvenile loggerhead turtle (Torrent et al. 2002). This microorganism is a pathogen of marine lobsters (Marks et al. 1992) and it has also been isolated from fishes in the Atlantic Ocean (Menezes 1992). The majority of reports documenting *Citrobacter* infections in reptiles involve terrestrial chelonians (Jacobson 2007). However, *Citrobacter* sp. was isolated from 4 loggerhead turtles with systemic lesions (Orós et al. 2005).

Glazebrook & Campbell (1990a) reported *Pseudomonas* sp. infections in the salt glands of 2 farmed green sea turtles *Chelonia mydas* due to the removal of foreign material from the main excretory duct leading from the posterior orbit. The procedure involved the

use of forceps which introduced bacteria and led to infection.

The mean values for plasma concentrations of sodium and chloride and plasma osmolality from our 25 healthy loggerhead turtles were similar to those previously reported for this species (Lutz & Dunbar-Cooper 1987, Kakizoe et al. 2007). The very high individual plasma concentrations of sodium and chloride, and plasma osmolality from 2 loggerhead turtles suffering from salt gland adenitis were caused by the misfunction of the salt glands due to the severe lesions observed in these organs. Effects of storage time and temperature after blood sampling from turkeys on plasma concentrations of potassium, sodium, and chloride have been previously reported (Reece et al. 2006). However, there was no variation in storage time between healthy and diseased turtles. Although sea turtles show some plasticity in their internal ionic composition (Lutz & Dunbar-Cooper 1987, Reina & Cooper 2000, Casal & Orós, 2009), they maintain homeostasis in a desiccating environment (Reina et al. 2002). These cases suggest that failure in maintaining homeostasis due to severe lesions in the salt glands can cause stranding and/or death of sea turtles.

Acknowledgements. The authors thank P. Castro and A. Afonso, Department of Morphology, University of Las Palmas de Gran Canaria, for technical assistance. Funding for this paper was partially provided by the autonomic project PI 2007/044, ACIISI, Gobierno de Canarias.

LITERATURE CITED

Abel JH, Ellis RA (1966) Histochemical and electron microscope observations on the salt secreting lachrymal glands of marine turtles. Am J Anat 118:337–358

Casal AB, Orós J (2009) Plasma biochemistry and haematology values in juvenile loggerhead sea turtles undergoing rehabilitation. Vet Rec 164:663–665

Flint M, Patterson-Kane JC, Limpus CJ, Work TM, Blair D, Mills PC (2009) Postmortem diagnostic investigation of disease in free-ranging marine turtle populations: a review of common pathologic findings and protocols. J Vet Diagn Invest 21:733–759

Glazebrook JS, Campbell RSF (1990a) A survey of the diseases of marine turtles in northern Australia. I. Farmed turtles. Dis Aquat Org 9:83–95

Glazebrook JS, Campbell RSF (1990b) A survey of the diseases of marine turtles in northern Australia II. Oceanarium-reared and wild turtles. Dis Aquat Org 9:97–104

Glazebrook JS, Campbell RSF, Thomas AT (1993) Studies on an ulcerative stomatitis–obstructive rhinitis–pneumonia disease complex in hatchling and juvenile sea turtles *Chelonia mydas* and *Caretta caretta*. Dis Aquat Org 16: 133–147

Gordon AN, Kelly WR, Lester JG (1993) Epizootic mortality of free-living green turtles, *Chelonia mydas*, due to coccidiosis. J Wildl Dis 29:490–494

Jacobson ER (2007) Bacterial diseases of reptiles. In: Jacobson ER (ed) Infectious diseases and pathology of reptiles. CRC Press, Boca Raton, FL, p 461–526

Kakizoe Y, Sakaoka K, Kakizoe F, Yoshii M, Nakamura H, Kanou Y, Uchida I (2007) Successive changes of hematologic characteristics and plasma chemistry values of juvenile loggerhead turtles (*Caretta caretta*). J Zoo Wildl Med 38:77–84

Lutz PL, Dunbar-Cooper A (1987) Variations in the blood chemistry of the loggerhead sea turtle, *Caretta caretta*. Fish Bull 85:37–43

Marks LJ, Stewart JE, Hastein T (1992) Evaluation of an indirect fluorescent antibody technique for detection of *Aerococcus viridans* (var.) *homari*, pathogen of homarid lobsters. Dis Aquat Org 13:133–138

Marshall AT, Cooper PD (1988) Secretory capacity of the lachrymal salt gland of hatchling sea turtles, *Chelonia mydas*. J Comp Physiol B 157:821–827

Menezes J (1992) Hazards from pathogens carried by wild fish particularly wrasse used as lice cleaners. Bull Eur Assoc Fish Pathol 12:194–195

Nicolson SW, Lutz PL (1989) Salt gland function in the green sea turtle *Chelonia mydas*. J Exp Biol 144:171–184

Orós J, Torrent A, Calabuig P, Déniz S (2005) Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998–2001). Dis Aquat Org 63: 13–24

Owens DW, Ruiz GJ (1980) New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36:17–20

Paré JA, Sigler L, Rosenthal KL, Mader DR (2006) Microbiology: fungal and bacterial diseases of reptiles. In: Mader DR (ed) Reptile medicine and surgery, 2nd edn. Saunders Elsevier, St. Louis, MO, p 217–238

Reece WO, Trampel DW, Koehler KJ (2006) Effects of storage time and temperature after blood sampling from turkeys on plasma concentrations of potassium, sodium, and chloride. Poult Sci 85:1095–1097

Reichenbach-Klinke H, Elkan E (1965) The principal diseases of lower vertebrates. Diseases of reptiles. Academic Press, London

Reina R (2000) Salt gland blood flow in the hatchling green turtle, *Chelonia mydas*. J Comp Physiol B 170:573–580

Reina RD, Cooper PD (2000) Control of salt gland activity in the hatchling green sea turtle, *Chelonia mydas*. J Comp Physiol B 170:27–35

Reina RD, Jones TT, Spotila JR (2002) Salt and water regulation by the leatherback sea turtle *Dermochelys coriacea*. J Exp Biol 205:1853–1860

Roberts RJ (1978) Fish pathology. Bailliere Tidall, London

Torrent A, Déniz S, Ruiz A, Calabuig P, Sicilia J, Orós J (2002) Esophageal diverticulum associated with *Aerococcus viridans* in a loggerhead sea turtle (*Caretta caretta*). J Wildl Dis 38:221–223

Wolke RE, George A (1981) Sea turtle necropsy manual. NOAA Tech Memo NMFS SEFC-24

Work TM, Balazs GH, Wolcott M, Morris R (2003) Bacteraemia in free-ranging Hawaiian green turtles *Chelonia mydas* with fibropapillomatosis. Dis Aquat Org 53:41–46

Work TM, Balazs GH, Rameyer RA, Morris RA (2004) Retrospective pathology survey of green turtles *Chelonia mydas* with fibropapillomatosis in the Hawaiian Islands, 1993-2003. Dis Aquat Org 62:163–176

Wyneken J (2001) The anatomy of sea turtles. NOAA Tech Memo NMFS SEFSC-470

Editorial responsibility: Alex Hyatt, Geelong, Victoria, Australia

Submitted: November 1, 2010; Accepted: February 28, 2011
Proofs received from author(s): May 16, 2011