

Department of Primary Industry and Resources
Berrimah Veterinary Laboratories

POSTAL ADDRESS
GPO Box 3000
Darwin NT 0801

GENERAL RECEPTION PHONE: 08 89992249
Fax: 08 89992024

DELIVERY ADDRESS
29 Makagon Road
Berrimah NT 0828

20160855

FINAL Report Version: 1

Print Date: 03/10/2016

Accession number: 20160855 **Property/Location/Clinic details:** TIWI ALT (MELVILLE ISLAND)
BVL SAN: B50917 **Property Id Code (PIC):** TJDG0392
Animal Owner: Melville Island - Loc 11.8255; 131.1162
Date collected: 29/08/2016 **Date received:** 31/08/2016

Submitter: NTG Ms. Rachel Groom
Phone: 0409813479
Fax:
Email: rachel.groom@nt.gov.au

Animal Type: TURTLE **Common name:** N/A
Breed: N/A **Scientific name:** N/A
Sex: Female **Age:** 0 Mature **NLIS Tag**

Duty Pathologist for this case: Kitman Dyrting **Department Contact Officer:** Dr. Elizabeth Stedman
Reports To: Ms. Rachel Groom

Case History: 20160855

One mature wild Loggerhead turtle, *Caretta caretta*, carcass was submitted for post mortem examination late in the afternoon of 29/08/2016. The submitter requested the tracker and a range of other samples to be saved for research purpose.

Pathology: 20160855

Animal ID: Turtle **Specimen:** Body

Gross Pathology

This was the carcass of the mature, female, Loggerhead turtle, *Caretta caretta*, weighed approximately 56 kg (calculated by subtracting gross weight from weights of electronic tracker, chains of hoist, the blue tarp and duct tape). Other morphometric measurements were as listed.

- Carapace length (curved): 91 cm
- Carapace length (straight): 86.5 cm
- Carapace width (curved): 81 cm
- Carapace width (straight): 65 cm
- Plastron length: 60.4 cm
- Plastron width: 66 cm
- Plastron to tail tip (from posterior plastron): 16.5 cm



NATA Accredited Laboratory
Number 13626
Accredited for compliance with ISO/IEC17025 - Testing
This document shall not be reproduced except in full.

3-Oct-2016



* NATA accreditation does not cover the performance of this test.

- Plastron to vent (from posterior plastron): 11 cm
- Head length: 20.4 cm
- Head width: 19 cm

Identification

Attached tightly onto the midline of the carapace cranially, there was a device approximately 5 cm x 5 cm x 2 cm with an antenna about 15 cm long (electronic tracer). The tracker had been fixed in place by red-coloured epoxy glue which covered approximately 10% of the carapace surface. Removal of the tracker and dried epoxy glue revealed no apparent damage to the underlying scutes or internal bony structure of the carapace. (Submitter had removed flipper tags from forelimbs before carcass submission.)

Carcass condition

The carcass condition was fair (degree of decomposition was about 3 out of a scale of 6). There was no significant gas release on incision into the plastron and the internal organs were generally intact.

Nutritional condition

The nutritional condition of the turtle was very poor. It was emaciated with dehydration. Both eyes were markedly sunken into the eye sockets. The plastron was sunken, concave and pliable. The scutes of the plastron of the ventral midline seam did not meet tightly along the midline but held together by thick connective tissue which was more pronounced cranially. (The distance between the scutes along the midline seam ranged from 1 cm cranially to 0.4 cm caudally.) Internally in the plastron, the bony projections on the medial edges of the hyoplastron and hypoplastron could easily be palpated as sharp projections. There were small amounts of musculing and subcutaneous fat associated with the limbs and plastron. The skeletal muscles were generally pale and flabby (marked muscle atrophy). The visceral fat was dark greenish grey with a watery consistency (marked serous atrophy of fat).

External condition

The entire external surface of the carapace was covered in dried algae that had obscured all the scutes. In addition, there were generally low numbers of gooseneck barnacles and acorn barnacles attached on the carapace and plastron.

The caudolateral part of the carapace over the right hind limb was missing. This defect was half oval in shape, with the widest point at the most lateral edge of the carapace, about 22 cm long by 12 cm deep to the lateral edge. The edges of the defect were smooth and rounded (old and healed wound) and there was no penetrating wound into the coelomic cavity. On the rest of the carapace superficially, there were about half a dozen randomly scattered linear scratch marks, ranging from 2 cm to 15 cm long by about 2 mm deep. There was no disruption to the underlying bony structure of the carapace.

The left front flipper had been amputated distally at the level of the first carpal joint. It was estimated that approximately 30 cm of the distal flipper was missing. The end of this amputated limb had healed and completely covered in skin. There was no evidence of infection or inflammation in the underlying bone.

Both the left hind foot and the right hind foot had a deep, full thickness, relatively large wedge- shape defect distally, approximately 4 cm deep to the edge by 5.5 cm along the edge and 4 cm deep to the edge by 12 cm along the edge respectively. The edges of both defects were generally smooth and rounded with no evidence of inflammation or infection, but the normal rudder- shape outline of both hind feet had been destroyed. (Both Rachel and Aud commented verbally that the right feet lesion was caused by entangled fishing line which had been removed when this turtle was seen nesting late last year.)

Internal findings

In the muscle of the left pectoral girdle, there was an approximately 1 cm circular, slightly reddened, depressed focus with a sharply demarcated central yellowish brown necrotic centre which was about 3 mm deep. This focus of necrosis was likely caused by direct bony trauma from one of the medial bony projections on the right medial hyoplastron when apposed (as a result of emaciation and lack of soft tissue cushioning there).

In the respiratory tract, the lumen of the entire trachea and lower airways in the lungs contained a large amount of stable foam, indicating severe diffuse pulmonary oedema. Both lungs were diffusely greyish brown. In the soft tissue of the mediastinum near the tracheal bifurcation, there was a mild haemorrhage.

In the pericardial sac, there was a moderate amount, about 50 ml, of clear reddish brown fluid. In the anterior ventricle of the heart, near the base of the pulmonary trunk at the free edge of a septal endocardium (probably of cavum venosum), there was a 1 cm diameter by 2 mm area of tissue thickening which was yellowish, translucent and myxomatous with smooth outline (# see comment). Adhering loosely elsewhere on the endocardium of the ventricle and atrium, there were several trematodes, about 1 mm wide by 4 mm long, presumptive Spirorchiid flukes, however, there were no associated damages in the heart or major blood vessels.

The thyroid gland was large, about 3 cm in diameter by 6 cm long, slightly nodular, orange-brown, translucent and gelatinous on cut surface.

In the coelom cavity, there was a large amount, about 3 L, of dark brownish green fluid. The mesentery appeared to be twisted and wrapping around the colon, however, the mesenteric blood vessels or the intestinal serosal blood vessels were not engorged, there was no adhesion and the twist could easily be reduced upon removal of the gastrointestinal tract. These additional observations supported the twist in the mesentery was an post-mortem artefact. Bile imbibition was present along the length of the gastrointestinal tract, part of the liver and mesentery (post-mortem changes).

The liver was diffusely dark brownish purple, firm, smaller than normal with sharp edges (liver atrophy). The gall bladder was engorged by a large amount of dark green, slightly viscous bile.

The spleen appeared normal apart from several randomly scattered small, about 1 mm in diameter, slightly raised spots, presumptive granulomatous responses to Spirorchiid eggs. In the nearby mesentery, there were 3 accessory spleens about 0.5 cm to 1 cm in diameter. Similarly there were a few dark spots in the accessory spleens.

The tongue, oral cavity and oesophagus were unremarkable. The stomach and upper intestine was empty. The large intestine was extended by a moderate amount of gas and a small amount of bile stained fluid (post-mortem changes). The lower intestine, colon and rectum, contained a small to moderate amount of crushed calcareous shells, a few sea urchin spines and some unidentified sponge-like material.

The kidney surface was mildly diffusely congested. The urinary bladder did not contain urine but the mucosa had a small amount of yellowish brown mucus, mild petechiae and a few loosely attached unidentified trematodes, approximately 2 mm wide by 3 mm long.

Both ovaries had numerous dark greenish atretic follicles, generally < 2 cm in diameter, along with numerous small yellowish-white corpus albicans, about 1 to 2 mm in diameter. The presence of corpus albicans indicated the turtle had nested previously. The oviducts were normal.

The brain and eyes were not examined.

Gross provisional diagnosis

- Emaciation
- Dehydration
- Severe acute diffuse pulmonary oedema
- Diffuse thyroid hyperplasia (colloid goiter)
- Multiple old injuries resulted in missing body parts including entire left front flipper, part of left and right hind feet and part of left caudolateral carapace
- Mild focal subacute muscle necrosis in the left pectoral girdle
- Mild mixed parasitic trematode infections (presumptive Spirorchiid blood flukes in heart and an unidentified fluke in urinary bladder) with presumptive granulomas in the spleen due to Spirorchiid eggs
- Focal endocardopathy, dubious (# see comment below)

Comment

The turtle carcass had been stored in the cold room until post-mortem examination was conducted in the morning of 31 /08/2016. Necropsy was performed by Kitman Dyrting and Ayril Harburn, in the presence of Racheal Groom (Department of Land Resource Management) and Ian Bell (Queensland Department of Environment and Heritage Protection) who assisted in collecting samples and taking pictures for research purposes.

The immediate cause of death of this turtle was severe pulmonary oedema which was likely resulted from terminal heart failure from exhaustion and hypovolaemia (dehydration).

The turtle was emaciated which was evidenced by marked muscle atrophy, serous atrophy of fat, liver atrophy and perhaps ovarian atrophy, although the latter (ovary condition) could also be affected by seasonality. The focus of muscle necrosis in the left pectoral girdle was likely secondary to emaciation. The excessive fluid accumulations in the coelomic cavity and the epicardial sac could be a combination of malnutrition (hypoproteinaemia) and post-mortem autolysis. This turtle had concurrent multiple healed but significant injuries. Most importantly the missing left front flipper blade and the misshaped hind feet would have affected the normal swimming behaviour and hunting ability of this turtle, especially if all these happened before the turtle had sufficient time to adapt to each of the injuries. Apart from the injury in the right hind feet (with entangled fishing line which had been removed late last year), the causes of other old injuries were unknown. The enlarged thyroid gland in this case could be a response to hypofunction of the gland secondary to chronic debilitation or illness. The brain and eyes were not examined as the research team requested the entire head to be returned.

A range of tissue samples, including the dubious heart lesion (#), have been collected and preserved for histopathology to look for underlying microscopic disease processes, although the state of moderate to severe carcass autolysis would affect interpretation of findings. Further testing, such as microbiology, will be conducted on other stored tissue samples if indicated. Di Barton (Department of Primary Industry and Fisheries) is assisting with parasite identification.

The electronic tracker, dried epoxy glue, skin samples, one humerus, epibiont samples, intestinal contents, ovaries and the head were collected for the research team for further examination.

Pathology: 20160855

Animal ID: Turtle

Specimen: Necropsy Tissues

Histopathology

Tissues are in moderate to severe autolysis. Tissues examined include skeletal muscle, fat, liver, spleen, pancreas, heart, large artery, lung, thyroid gland, kidney, urinary bladder, ovary, oviduct, stomach, small intestine, large intestine and spinal cord.

Skeletal muscle (3 sections):

Diffusely the diameters of the muscle fibres are generally very small (generalised severe muscular atrophy) with no associated inflammation or peripheral neuropathy. The focus of muscle necrosis in the left pectoral girdle noted grossly was necrotic tissue surrounded by many granulocytes with lesser numbers of histiocytes and fibroblasts. Gram stain failed to reveal bacteria. (However, the number of infiltrating granulocytes appeared to be in excess of normal muscle healing response, suggestive of local granulocyte chemotactic response such as due to transient bacterial infection or bacterial toxin.)

Fat (1 section):

Diffusely the adipocytes are generally small with little fat vacuoles but prominent round nuclei. There is no inflammatory response but there are moderate to large numbers of resident melanomacrophages.

Liver (2 sections):

Diffusely, the hepatocytes are small (atrophy) and contain low to moderate amount of fine brown cytoplasmic pigment which is Perl's stain positive (haemosiderin). In the hepatic sinusoids, there are many Kupffer 's cells containing abundant granular dark-brown cytoplasmic pigment which is also Perl's stain positive (haemosiderin). There is no inflammatory response.

Spleen (3 sections):

Multifocally there is moderate numbers of well-encapsulated granuloma containing many yellowish-brown, angular, thick-walled Spirorchiid trematode eggs (Spirorchiid granuloma). In the parenchyma diffusely, there are many macrophages containing abundant granular dark-brown cytoplasmic pigment which is Perl's stain positive (haemosiderin).

Pancreas (1 section):

Diffusely the exocrine pancreatic acinar cells are small and do not have discernible cytoplasmic zymogen granules. Randomly, there are a few well-encapsulated Spirorchiid granulomas.

Thyroid gland (2 sections):

The thyroid follicles are generally very large and distended with a large amount of homogeneous eosinophilic thyroid colloid, however, the follicular cells are low cuboidal (inactive). Apart from a few small foci of mild infiltration of macrophages containing yellowish-brown fine cytoplasmic pigment (presumptive lipofuscin or ceroid lipopigment related to advancing age), there is no inflammatory response.

Large artery (carotid or subclavian, 2 sections):

In both sections, the intimal layer is regionally oedematous with moderate increase in cellularity (intimal hyperplasia). In one section, extending from the intima to the media there is a focus of moderate to severe granulocytic inflammation with a few multinucleated giant cells and fibrin deposition.

Heart (4 sections):

In the vascular spaces in the myocardium, there are very occasional Spirorchiid trematode eggs with nil to generally mild inflammatory response. The dubious focus of "endocardiopathy" noted grossly is confirmed to be an area of normal cartilaginous structure with moderate amount of extracellular matrix.

Lung (4 sections):

There is moderate diffuse vascular congestion. As in the heart, there are a few Spirorchiid eggs associated with generally nil to mild inflammatory response.

Kidney (1 section):

There is patchy vascular congestion. As in the heart and lung, there are a few Spirorchiid eggs associated with generally mild inflammatory response.

Urinary bladder (1 section):

Multifocally there is very mild perivascular lymphohistiocytic infiltration and scattered very mild haemorrhage. (The unidentified intraluminal trematodes did not cause significant inflammatory response.) As in the kidney, there are a few Spirorchiid eggs associated with generally mild inflammatory response.

Stomach (1 section) and intestine (7 sections):

Multifocally extending from the lamina propria to the serosa, there are mild to moderate well-encapsulated Spirorchiid granulomas. The mucosa is sloughed (due to autolysis) and could not be assessed.

Ovary (1 section):

There are several atretic follicles that are infiltrated with foamy macrophages, several normal looking small follicles and a collagenous corpus albican with mild mineralization.

Oviduct (1 section):

There is no specific finding.

Spinal cord (cervical, 1 section):

There is no specific finding.

Histological diagnosis

- Skeletal muscle: Severe diffuse muscular atrophy; Incidental focal chronic pyogranulomatous necrotising myositis
- Fat: Diffuse serous atrophy of fat
- Liver: Diffuse hepatocyte atrophy; Moderate hepatic haemosiderosis
- Spleen: Moderate splenic haemosiderosis; Multifocal moderate Spirorchiid egg granuloma
- Pancreas: Diffuse depletion of zymogen granules in exocrine pancreatic cells
- Thyroid gland: Diffuse quiescent colloid goiter
- Artery: Focal subacute moderate pyogranulomatous endarteritis

Pathology: 20160855

- Heart: Multifocal mild Spirorchiid egg granuloma
- Lung: Multifocal mild Spirorchiid egg granuloma
- Kidney and urinary bladder: Multifocal mild Spirorchiid egg granuloma
- Stomach and intestines: Multifocal mild to moderate Spirorchiid egg granuloma

Comments

There are no underlying microscopic disease processes, such as sepsis, overwhelming inflammation or neoplasia. However, there are compelling histological evidences that the turtle in this case suffered from marked generalised tissue wasting (muscular atrophy, serous atrophy of fat, atrophy of hepatocytes and reduced zymogen granules in exocrine pancreatic cells) consistent with emaciation caused by severe malnutrition and starvation. As noted grossly, the condition was most probably resulted from severe physical debilitation from the multiple limb injuries. The thyroid gland was enlarged but quiescent indicating the gland was in hypofunction state. Splenic and hepatic haemosiderosis indicated there was iron sequestration. Both changes were likely secondary to chronic illnesses related to malnutrition and starvation.

In the lung, the lack of eosinophilic fluid in the lower airspaces indicates the pulmonary oedema noted grossly was low-protein pulmonary oedema. There is no pneumonia. There are no foamy macrophages in the airspaces to suggest chronic heart failure. The pulmonary oedema noted grossly was confirmed to be acute and was a terminal finding.

Spirorchiid egg granulomas were found in various tissues, but they were well-encapsulated and did not appear to have affected normal tissue functions. There was a focal subacute endarteritis, suspected to be inflicted by intraluminal Spirorchiid trematodes (a few of these parasites were found in the heart grossly), but there was no associated thrombosis or vascular rupture to suggest compromised cardiovascular function. The Spirorchiid trematode burden was generally considered moderate, probably with limited clinical significance.

Case Summary: 20160855

31 Aug to 2 Sep 2016: Interim gross findings were discussed verbally and via email exchanges.

19 Sep 2016: Interim report-1. Note written Gross findings and comments. Histopathology is pending.

03 Oct 2016: Final report-1. Note Histopathology findings and comments.



Dr Kitman Dyrting - Veterinary Pathologist

03/10/2016