

First Post-mortem report on a dolphin from West Lakes Shore



Nov 2021

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Animal no.: SAM accession number 21.024, University of Adelaide number 21-01943

Collection circumstances:

19 July 2021. The dead dolphin was reported by member of public on the South of Mirani court, West Lakes Shore. It was collected by marine parks rangers (Department of Environment and Water) on the same day. It was delivered to the Bolivar Facility and placed in a cold room for 4 days then in locked a freezer due to the COVID lockdown.

Location of carcass collection: South of Mirani court, West Lakes Shore 34° 51' 58.5''S, 138° 28' 41''E

Species: Indo-Pacific bottlenose dolphin (*Tursiops aduncus*),

Age: Adult.

Sex: female

Weight: 82.5 kg

Total length: 208 cm

Clinical history

No clinical history is available. Four swab samples were taken from its oral cavity, blowhole genital slit and anus for the culture and sensitivity test (bacterial test). *Vibrio alginolyticus* was found from all, *Vibrio parahaemolyticus* was found from the anus. No morbillivirus infection was evidenced.

Post-mortem:

Preparation: 10 August 2021. The dolphin was put into the macerating building to thaw out in preparation for the post-mortem the following week.

Date: 17 Aug 2021 (0830 to 1130 hrs) and

Examiners:

Gross findings of the post-mortem

The condition of the carcass was assigned to Geraci Code 2 (skin intact, skin colour good, pancreas solid and other organs firm and intact).

External observations

Body condition of the dolphin was very emaciated with four features of emaciation (Kemper et al. 2016). Tattoo like skin lesion was observed near the blowhole. Several sets of tooth rakes were found on the posterior ventral surface.

One penetrating wound was observed on part of genital slit with redness and edema.

Internal observations

A thorough examination of all major body organs was carried out.

Focal subcutaneous haemorrhaging was observed on left side on the head and along the vertebrae.

The abdominal cavity contained large amount of fluid coloured with blood. Fibrous miliary nodules were spread on mesentery (including omentum), peritoneum and diaphragm. Gastric, mesenteric lymph nodes and the spleen were enlarged and swollen. A large abscess had formed a capsule on the external stomach wall. Pyogenic materials were found inside the uterus.

Adrenals were also enlarged and swollen.

In the thorax, the pericardium (visceral) near the origin of the left coronary artery showed haemorrhagic change. The lungs did not show any significant change, only partially collapsed in both anterior lobes. However, a moderate amount of froth was seen from trachea to bronchi in the lungs.

Meningeal congestion/ hyperaemic has also observed around the brain.

Severe trematode infections were found in the bilateral sinuses and in the soft tissues around the ear bones.

Histopathology findings of the post-mortem

Liver, kidney, spleen, pancreas, adrenals, lymph nodes, lungs, heart, skin, oral mucosa, spinal cord and brain tissue samples were prepared for histopathology examination.

All tissues show moderate to sometimes marked autolysis and freeze thaw artefact.

Oral mucosa: Mild irregular hyperplasia with mild segmental hyperkeratosis. There is superficial segmental mild accumulation of eosinophilic material between keratinocytes in the stratum corneum (oedema, protein). Diffuse surface bacteria but no host response (surface contaminants).

Skin, viral lesions, caudal to blow hole: There is superficial segmental mild accumulation of eosinophilic material between keratinocytes in the stratum corneum (oedema, protein), as well as hydropic degeneration of stratum spinosum keratinocytes, and rare intracytoplasmic hyaline intensely eosinophilic globular material (query pox viral inclusions). There is segmental separation of the epidermis from the dermis with formation of large clear vacuoles in the clefts, delineated by fibroblasts and low variable infiltration of neutrophils, lymphocytes and macrophages.

Liver: Diffusely, the periportal interstitium is infiltrated by mild to moderate numbers of inflammatory cells (lymphocytes, neutrophils, macrophages). The capsule is segmentally expanded by eosinophilic material (query fibrin) and low numbers of mixed leukocytes.

Mesenteric lymph node: The mesothelial surface covering the lymph node capsule is hyperplastic, infiltrated by mixed leukocytes, and covered by a thin layer of intensely eosinophilic fibrillar to compacted material (fibrin). Lymph node is suspected to be hyperplastic however cell preservation is very poor.

Kidney: No significant findings (subtle findings may be masked).

Spinal cord: scattered mild perivascular mononuclear infiltrates.

Lung: Diffuse flooding of alveoli by protein rich translucent eosinophilic fluid (autolytic vs. agonal change, vs pulmonary oedema). Bronchial mucosa is extensively lost (autolysed) however in regions is often replaced by refractile deeply basophilic crystalline material (mineral).

Spleen: the spleen is hypercellular with increased density and number of lymphocytes (reactive hyperplasia) and frequent megakaryocytes (extramedullary haematopoiesis). The splenic capsule is focally thickened by fibrosis, with bands of fibrosis dissecting around large extracellular aggregates of refractile crystalline intensely yellow to brown material (query foreign material vs. haematoidin and haemosiderin. Material is not birefringent under polarised light. Bands of collagen surrounding crystalline aggregates are frequently mineralised.

Skin genital slit area: There is focal loss of the epidermal layer, with well developed dense connective tissue in the underlying dermal layer.

Left lateral tail lesion: no significant findings

Skin, dorsal fin, cranial: – NSF Possible interesting change in interstitial tissue of muscle layer

Mid dorsal tail lesion: No Significant Findings (NSF)

Slit lesion: focally extensive epidermal erosion, superficial surface bacterial colonies (secondary invaders)

Heart: Rare scattered interstitial infiltrates of lymphocytes, plasma cells and rare neutrophils.

Diaphragm: On the abdominal surface the mesothelium is lost (artefact) however the surface is rarely covered by fibrin, and the sub mesothelial stroma is multifocally infiltrated by mixed leukocytes.

Blow hole: NSF

Adrenal gland: rare medullary vessels contain fibrin thrombi, otherwise NSF

Uterus: NSF

Right lateral thorax lesion: There is focally extensive fusion and anastomoses of rete pigs, with mild superficial dermal fibrosis subjacent to the dermo-epidermal junction (healed lesion scar? Dysplasia?)

brain, cerebrum, mid brain, cerebellum and brain stem: In all sections there is freeze thaw artefact and moderate to sometimes marked autolysis. Throughout all sections there is multifocal sporadic mild to occasional moderate perivascular mononuclear infiltrates (perivascular cuffing).

MICROSCOPIC DIAGNOSES

1. *Skin, viral lesions: locally extensive keratinocyte ballooning degeneration and intracorneal oedema with putative intrakeratinocyte intracytoplasmic inclusion bodies (putative poxviral dermatitis)*
2. *Brain and spinal cord: rare to mild non-suppurative encephalitis and myelitis*
3. *Heart: mild multifocal non-suppurative myocarditis*
4. *Liver: moderate periportal mixed chronic-active hepatitis*
5. *Lung: diffuse pulmonary oedema (DDx agonal or autolytic change), bronchiolar epithelial mineralisation*
6. *Diaphragm: fibrinosuppurative peritonitis*
7. *Spleen: focal capsular fibrosis and histiocytic and lymphoplasmacytic inflammation with suspected haematoidin and haemosiderin crystal deposition (DDx foreign material, other)*

COMMENT

A mild non-suppurative encephalitis and myelitis was observed across multiple sections of the brain and in the spinal cord. More subtle findings may have been hindered by freeze-thaw artefact and post mortem change. Causes of encephalitis in cetaceans include Morbillivirus, Herpesvirus, *Toxoplasma gondii*, *Brucella* sp., and *Nasitrema* sp. Morbillivirus testing was negative for this individual. *Brucella* serology for this individual is pending (VDL).

Toxoplasma serology revealed seropositivity implying previous exposure to the parasite. Chronic inflammation in the nervous system and heart (see below) may also reflect chronic subclinical toxoplasmosis. Herpesvirus PCR testing is available and is under investigation.

Histopathological changes in skin sections grossly suspected to have viral skin disease are histopathologically consistent with cetacean poxviral aetiology.

Changes in the diaphragm are consistent with septic peritonitis. Periportal hepatitis likely reflects non-specific change due to systemic (peritonitis) and gastrointestinal (gastric wall

abscess) inflammation. Lung changes are likely confounded by autolysis and free thaw artefact, however pulmonary oedema is strongly considered, such as may occur secondary to septic shock. Changes in the spleen capsule are uncertain, but suggest a focus of chronic haemorrhage, inflammation and scarring. The crystalline material is reminiscent of haematoidin, however other identity cannot be excluded. There is a mild interstitial myocarditis in the heart also, suggesting chronic inflammation.

Other tests

Bacterial tests : Table 1 summarises the bacteria identified. Samples were taken from the blowhole, oral cavity, faeces, skin of the genital area and stomach abscess.

Table 1: Bacterial test results for the dolphin (21.024, 21-01943)

Organ	Bacteria
blowhole	<i>Vibrio alginolyticus</i>
oral cavity	<i>Vibrio alginolyticus</i>
faeces	<i>Vibrio parahaemolyticus</i>
genital area skin	<i>Vegococcus fluvialis</i>
Stomach abscess	<i>Pseudomonas lundensis</i>

The stomach abscess was negative for acid fast bacilli which includes *Mycobacterium tuberculosis*.

Abdominal fluid cytology: neutrophil exudate and septic, it is consistent with septic suppurative peritonitis.

Toxoplasmosis: The serum test for toxoplasma was positive at 1000X dilution.

Morbillivirus: The PCR test was negative for lung tissue.

Brucellosis: Testing in progress.

Skeleton pathology: will be examined when the skeleton is prepared.

Heavy metal and Organic choline testing: will be organised.

Summary

The Circumstance of death categories developed by the South Australian Museum include five classifications and after post-mortem animals are assigned to one of these based on the information available. We conclude that the most likely circumstance for the dolphin from West lakes Shore is 'Disease (chronic peritonitis with secondary infection)'. The following is the evidence for this conclusion.

General health condition

The dolphin's body condition appeared to be very emaciated, suggesting that its death was the result of a chronic condition.

Skin lesions

Skin lesions observed near the blowhole were grossly suspicious for viral skin disease and histologically consistent with the etiology of cetacean pox virus. This lesion is called tattoo-like skin lesion and has been observed previously in SA waters (Kemper and Tomo, 2019). It is seen when the underlying physical conditions are impaired.

Abdominal organs

The most significant pathological findings of this dolphin were well capsulised abscesses on the gastric wall (externally) and disseminated miliary nodules on the peritoneal diaphragm and mesentery. The histology features of which are consistent with septic peritonitis.

Abdominal fluid cytology also suggested septic suppurative peritonitis.

The size of the abscess and the nature of its contents suggested a chronic inflammatory process, which appeared to be responsible for the peritonitis.

Aetiology of the abscess for this case is unknown.

A possible aetiology is the initiation of infection by perforation of the gastrointestinal wall. A previous case was seen on the SA coast (Brighton beach) where a catfish spine had penetrated the wall of the digestive tract. (Tomo and Kemper, 2012).

Liver had mild to moderate periportal fibrosis with hepatocellular atrophy, which often results from parasitic infection (Cowan 2002). Trematodes are often observed in the livers of SA dolphins, but the parasites themselves were not identified during this examination.

Thoracic organs

Mild inflammation was seen in the heart and lungs, but this was minor compared to that seen in the abdominal cavity and was probably secondary.

Cranial

A mild non-suppurative encephalitis and myelitis was observed in the brain and the spinal cord.

Toxoplasma infection was seropositive at 1000-fold dilution, suggesting possible reactivation in this dolphin. Toxoplasma is known to cause inflammation of the brain and muscles of dolphins, when the host's body condition is compromised. Severe trematode (*Nasitrema* sp.) infection was also observed surrounding soft tissue of the ear and the pterygoid air sinuses. Trematodes (*Nasitrema* sp.) infection of the skull has been known in various species of dolphins worldwide since the 1990s (O'Shea et al 1991, Díaz-Delgado et al 2018, Lim et al. 2016). Infection of this parasite in the soft tissues around the ear bone was also observed in SA water during the UME (Kemper et al 2016).

Conclusion

The conclusion is that the chronic inflammation seen in the abdominal cavity caused the dolphin's condition to deteriorate and that these parasites be infected secondary. In addition, the peritonitis, probably caused by a bacterial infection, led to septicaemia and death.

Literature cited

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