Post-mortem report on Talulla, an ADS dolphin

Oct 2021

Animal no.: SAM accession number 21.029, University of Adelaide number 21-02044

Collection circumstances:

21 Aug 2021. The dead dolphin was reported by Garden Island Yacht Club, at Port River, Adelaide Dolphin Sanctuary. It was picked up by marine parks rangers (Department of Environment and Water) on the same day. It was delivered to the Bolivar Facility of SA Museum and placed in a cold room for 3 days.

Location of carcass collection: Angas Inlet, Garden Island 34° 48' 9''S, 138° 31' 58''E

Species: Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), identified by specialist, based on external features.

Age: adult

Sex: male

Weight: 70.3 kg

Total length: 197cm

Clinical history

Tallula was born in March 2009 to female ADS dolphin Wave. Tallula and his mother Wave had severe extensive burn-like skin lesions in April 2010 which healed by 2014 (Bossley and Woolfall, 2014).

He had been observed tossing fish scraps and appeared to have a good appetite with good body condition in early June 2021. The first change in his body condition was observed on 5 August 2021, after which he became emaciated. He was last seen alive on 19 August 2021.

Post-mortem examination:

Date :24 August 2021.

Sample collection: blowhole, mouth and rectum were swabbed and used for bacterial and fungal examination.

Measurements and images: The dolphin's external images and measurements were taken, and morphological features were recorded.

Necropsy: 25 Aug 2021 (0830 to 1230 hrs). Necropsy was conducted.

Gross findings of the post-mortem

The condition of the carcass was assigned to Geraci's Code 2 (carcass in good condition_ fresh) (Geraci and Lounsbury, 2005) according to the following observations. Skin intact with scavenger damage on right eye, skin colour good, pancreas solid and other organs were firm and intact.

External observations

Body condition of the dolphin was very emaciated, with four features of emaciation (Kemper et al 2016). Several sets of fresh tooth rakes were found on the left lateral and the ventral skin surface.

Internal observations

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

Body cavities: both abdominal and thoracic cavity contained a small amount of fluid with good organ position.

Mouth: the oral mucosa was observed to be thickened and ulcerated.

Stomach: foreign bodies (fishing hook and fishing line) were found in the first chamber of the stomach. Micro erosions dispersed on the stomach and oesophagus mucous membrane. Stomach contained a small amount of food.

Liver: mildly congested.

Spleen: slightly atrophied with dark spots on the surface. Cut surface showed no significant change.

Adrenals: mildly enlarged.

Lungs: no significant change. However, a moderate amount of froth and viscous blood coloured fluid was observed from trachea to bronchi in the lungs.

Brain: meningeal congestion/hyperaemia was present.

There were no significant gross findings in other organs.

No severe subcutaneous haemorrhaging was observed in the whole body.

MACROSCOPIC DIAGNOSES

1. *Body condition: severely emaciated*
2. *Oral mucosa and blow hole: hypertrophic mucosa*
3. *Liver: mild congestion*
4. *Stomach: contamination by foreign materials*
5. *Brain: meningeal congestion/hyperaemia*

Histopathology findings of the post-mortem

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

All tissues show moderate to sometimes marked autolysis.

Thyroid gland. The gland is hypercellular with few colloidal follicles present. Interlobular stromal tissue is mildly increased. Review of published thyroid histopathology for dolphin species as well as review of archived museum thyroid histopathology in age matched animals did not show significant variation with the examined gland for Tallula.

Oral mucosa; hard palate: Oral mucosa is irregularly hyperplastic and segmentally ulcerated. There is marked surface bacterial overgrowth (post mortem contamination). In the irregularly hyperplastic mucosa there is fusion and anastomosing of rete pegs, and mild multifocal dysplasia in keratinocyte differentiation.

blow hole: In the epidermis/ mucocutaneous junction around the blow hole there is focally extensive hyperkeratotic mixed neutrophilic, histiocytic and lymphocytic dermatitis and epidermitis.

lung: diffuse congestion. There is multifocal mild flooding of alveolar lumina by eosinophilic translucent material (protein rich oedema), diapedesis and rare haemorrhage. Rare cross sections through nematodes within airways. Occasional post mortem invader bacteria present.

Liver: Mild diffuse congestion. Diffuse thinning of hepatocellular cords (atrophic change, inanition). Rare to mild periportal infiltrates and lymphocytes and plasma cells. In some sections there is moderate periportal to rarely bridging fibrosis

Spleen: moderate diffuse central lymphoid follicular lymphocytolysis/ depletion

Spinal cord: Evaluation and interpretation is hindered by autolysis. Query rare axonal spheroids in white matter, zonal distribution not evident. Scattered vacuolation of white and grey matter and rarely in neurons (interpreted as an artefact of autolysis).

No significant findings were found in the following organs: kidney, heart adrenal gland, skin and blubber injury, stomach, oesophagus, cerebellum, cerebrum, medulla, cerebral hemispheres

MICROSCOPIC DIAGNOSES

1. *Liver: mild to moderate periportal to rarely bridging fibrosis; diffuse hepatocellular atrophy*
2. *Oral mucosa and blow hole: hyperplastic mixed dermatitis, ulcerative, hyperkeratotic*
3. *Spleen: lymphoid follicular lymphocytolysis/ depletion*

Other tests

Urine tests (appendix 1): Sample was taken during post mortem examination. Protein urea, occult blood was strongly positive. Bilirubin was weakly positive. Granular casts, struvite and amorphous crystals were occasionally found. A few squamous epithelial cells were found. As the samples were taken during post-mortem examination, the results do not necessarily reflect the condition of the dolphin before death.

Bacterial tests (appendix 2): Table 1 summarises the bacteria identified. Samples were taken from blowhole, oral cavity, faeces, blood from heart, brain and cerebrospinal fluid.

Table 1: Bacterial test results for Tallula

Organ	Bacteria
blowhole	*Vibrio alginolyticus, Escherichia coli, Enterococcus faecalis, Erysipelothrix rhusiopathiae*
oral cavity	*Vibrio alginolyticus, Escherichia coli, Enterococcus faecalis, Erysipelothrix rhusiopathiae*
faeces	*Morganella morganii, Hafnia alvei, Vibrio anginolyticus, Phobacterium damselae subsp damselae*
blood (heart)	*Vegococcus fluvialis*
brain	*Vegococcus fluvialis*
cerebrospinal fluid	*Vegococcus fluvialis*

Some bacteria are known to cause opportunistic infections, but there are no histological results to support this. Therefore, these bacteria are considered as marine environmental organisms.
These bacteria species are the first time appeared in this region_ *Hafnia alvei, Vegococcus fluvialis and Erysipelothrix rhusiopathiae*

Toxoplasmosis: The serum test for toxoplasma was positive at 10X dilution.
Morbillivirus: PCR test was negative for lung tissue (appendix 3).
Brucellosis: Testing in progress
Skeleton pathology: will be examined when the skeleton have been prepared.
Heavy metal and Organic choline testing: will be organised.

Summary

We conclude that the most likely circumstance of death (Kemper et al. 2005) for the dolphin, Tallula, is 'Unknown'. The following is the evidence for this conclusion.

General health condition
The body condition of Tallula appeared to be very emaciated. This suggests that death was not acute. The liver histopathology findings supported that the animal was starving. There were no signs of significant injury, infectious disease, or neoplasia.

Foreign body in the stomach

A large size fishing hook and a fishing line were found in the first chamber of the stomach. Small amounts of small fish bones and small squid beaks were also found in the stomach contents. These hooks are usually used to catch large fish (e.g., mulloway). And as the bait for these larger fish is usually fresh small fish and squid, which are also food for bottlenose dolphins, it is likely that Tallula mistook one of these as his prey and ate it. Many small erosions were found on the mucosa from the oesophagus to the first chamber of the stomach. These gross changes were probably caused by physical stimulation by the fishing gear. However, microscopic observations showed no significant changes on these mucous.

Evidence of diseases

There was no severe sign of infectious disease nor any other diseases in any organs we examined.

Serological test was positive for toxoplasma infection at a 10-fold dilution, indicating that Tallulah had been exposed to toxoplasma during his life including before his birth. Toxoplasma has a wide range of intermediate host including dolphins. According to the host immune status, toxoplasmosis causes some symptoms. The seropositive level of toxoplasmosis is low, it is unlikely that this protozoan is actively effecting Tallulah's body condition at the time.

Mild lung nematode infection was found in lungs. Lung nematode infection has been observed since 2005 in SA waters (Tomo et al. 2010, Kemper et al. 2016). Severe lung nematode infections can be fatal, occupying a significant area of the lung and causing respiratory failure; in some cases the parasite itself brings secondary bacterial or fungal infections (Tomo et al. 2010). Alternatively, if the body condition has deteriorated for other primary reasons, lung nematodes may be a secondary infection. In the case of Talulla, this infection is quite mild and not fatal.

The mild lymphocytolysis/depletion of the spleen suggest that Tallula's immune system appeared to be mildly stressed because of its poor body condition.

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

Oral mucosa had hyperplastic and ulcerative dermatitis and the blowhole skin had hyperkeratotic dermatitis. Oral mucosal hyperplastic dermatitis can be caused by microorganisms. Papillomatosis in a blowhole has been reported in a dolphin in the ADS (Byard et al. 2010). The cause of this has not been determined yet.

Possible cause of emaciation and further investigation

Further examinations are underway of brucellosis test, skeleton pathologies and heavy metal levels in organs.

As no significant pathologies were found in Tallula, the primary cause of death remains unknown. Therefore in comparison with the reports from around the world of dolphin's unusual dolphin's mortalities, the following possibilities are considered.

Chemical exposure: Review of published thyroid histopathology for dolphin species as well as review of archived museum thyroid histopathology in age suggested Tallula's thyroid may be in normal range. The thyroid gland can be altered by exposure to chemicals. Among these, PCBs are known endocrine disruptors and are known to have hormone disrupting actions. US report found large yearly variability in the exposure of individual dolphins as well as marked differences in habitat usage within a year by individual dolphins, which, depending on the overlap with vessel activity, affected an individual's exposure (Mearns et al. 2016).

Food chain: Dolphins are probably one of the top predators in the Adelaide Dolphin Sanctuary. They might have ingested harmful substances that have accumulated in their habitat, e.g. the algae, micro-organisms, plankton, fish, crustaceans and squids in their area of residence.

An unusual mortality event in the Indian River Lagoon, USA in 2001, bottlenose dolphins (*Tursiops truncatus*) suspected to have been caused by ingestion paralytic shellfish toxins (Fire and VanDolah, 2012). Those dolphins were emaciated and displayed skin lesions, suggesting that multiple factors contributed to their overall poor health status (Bossart et al. 2003) (Broadwater et al. 2018). Saxitoxin from the dinoflagellate Alexandrium has been reported from (Hallegraeff et al. 1988) the Port River.

Squid is the common food of ADS bottlenose dolphins, but there is an evidence that they sometimes eat pufferfish as well (Byard et al. 2010). They are known to shift to other food sources when the amount of food available is limited.

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Appendix 1: urine test results

Risk Assessment
Risk: Not Specified **Sick:** Not Specified **Dec'd:** Not Specified **Rec. Arrival:** Not Specified

Urinalysis

Collection Method:	Not stated	
Volume:	3.0	mL
Colour:	Yellow	
Transparency:	Clear	

Chemistry

Specific Gravity (refrac):	1.019	
pH:	7.0	
Protein:	+++	
Glucose:	Negative	
Ketones:	Negative	
Urobilinogen:	0.2	
Bilirubin:	+	
Blood:	+++	

Microscopy

RBC/hpf:	<5	
WBC/hpf:	<5	
Fat:	Not Seen	
Sperm/hpf:	Not Seen	
Casts/lpf:	Granular	Occasional (1+)
Bacteria:	Not Seen	
Epithelial Cells/hpf:	Squamous	Few (2+)
Crystals:	Struvite	Occasional (1+)
Crystals 2:	Amorphous	Occasional (1+)

Appendix 2: bacterial test results

Risk Assessment
Risk: Not Specified **Sick:** Not Specified **Dec'd:** Not Specified **Rec. Arrival:** Not Specified

Microbiology

Microscopy: Gram Stain

Polymorphs Not Seen
Organisms NOT seen

Culture:
Light mixed growth (incl Vagococcus fluvialis)

Comment:
Heart blood swab

Risk Assessment
Risk: Not Specified **Sick:** Not Specified **Dec'd:** Not Specified **Rec. Arrival:** Not Specified

Microbiology

Microscopy: Gram Stain

Polymorphs Not Seen
Organisms NOT seen

Culture:
Light mixed growth (incl Vagococcus fluvialis)

Comment:
Brain swab

Collected: 24/08/21 00:00 **Subm.No:** 00128029 **Lab No.:** 21-51768207

Samples tested as received

FAECAL MC & S

Specimen: Faeces
Animal ID: 21.029

CULTURE
Faecal culture NEGATIVE for Salmonella sp.

Faecal culture NEGATIVE for Campylobacter sp.

Faecal culture NEGATIVE for Yersinia sp.

Heavy growth of Morganella morganii, Heavy growth of Hafnia alvei, Heavy growth of Vibrio alginolyticus, Moderate growth of Photobacterium damselae subsp damselae

Final Report
27/08/21

Your Ref: **OwnerID:** **Species:** Marine Mammal
Subm. No: **Lab No: 21-51768207** **DOB : N/A**
Samples tested as received All Tests Complete

MICROBIOLOGY **SPECIMEN:** Oral cavity swab
ANIMAL ID:21.029

MICROSCOPY
Numerous mixed bacteria. Numerous epithelial cells.

CULTURE
1. Heavy growth of Vibrio alginolyticus
2. Heavy growth of Escherichia coli
3. Light growth of Enterococcus faecalis
4. Light growth of Erysipelothrix rhusiopathiae.

COMMENT: Brucella species NOT isolated after 7 days incubation.

Final Report
01/09/21

Your Ref: **OwnerID:** **Species:** Marine Mammal
Subm. No: **Lab No: 21-51768207** **DOB : N/A**
Samples tested as received All Tests Complete

MICROBIOLOGY **SPECIMEN:** Blowhole swab
ANIMAL ID:21.029

MICROSCOPY
Numerous mixed bacteria. Numerous epithelial cells.

CULTURE
1. Heavy growth of Vibrio alginolyticus
2. Heavy growth of Escherichia coli
3. Light growth of Enterococcus faecalis
4. Light growth of Erysipelothrix rhusiopathiae.

COMMENT: Brucella species NOT isolated after 7 days incubation.

Final Report
01/09/21

Appendix 3: morbillivirus test result

RESULTS PH: 1300 307 190

Report Addressee:
VDL ROSEWORTHY
VDL DIAGNOSTIC LAB - ROSEWORTH
ROSEWORTHY CAMPUS-1454 MUDLA W
ROSEWORTHY 5371

Tested on 27/08/21
Reported on 01/09/21 14:00
Referred on 26/08/21 **by:**
VDL ROSEWORTHY
VDL DIAGNOSTIC LAB - ROSEWORTH
ROSEWORTHY CAMPUS-1454 MUDLA W
ROSEWORTHY 5371

Owner:
TURSIOPS ADUNCUS
SA MUSEUM NORTH TCE
ADELAIDE 5000

Animal/s: 21-02043
Marine Mammal
21.029
DOB: N/A

Collected: 26/08/21 00:00 **Subm.No:** 2151768346 **Lab No.:** 21-51768346

Samples tested as received All Tests Complete

REFERRED TEST

Disease/Test : Dolphin Morbillivirus
Method : Queensland 2010 Strain - TaqMan Assay
South Australia 2013 Strain - TaqMan Assay
Specimen Type: Fresh Lung
Animal ID : 21.029

RESULT : The sample submitted was Negative for both assays.

Comment : NATA/RCPA accreditation does not cover the performance of this service

This test was performed by: Australian Animal Health Laboratory(CSIRO)
NATA accreditation number: 13546