VDL PATHOLOGY NUMBER: 22-01721
STRANDING DATE/DATE FIRST SEEN: period of wasting/ weight loss, poor body condition first noted by 21/7/22, observed over previous month by volunteers and rangers
Animal seen alive? Yes, last seen alive– 1/8/22
RECOVERY DATE: 2/8/22, 4:25 pm 2/8/2022. Found upside down in approximately 1m deep water
STRANDING REPORTED BY: community member
EXAMINATION PERFORMED BY
DATE OF EXAMINATION: 3/8/22
LOCATION OF STRANDING: Ethelton – top of Port River adjacent Bower Road
Latitude: 34 51.11S
Longitude: 138 29.84E
WEATHER CONDITIONS AROUND TIME OF STRANDING: windy cold and wet

NUMBER OF ANIMALS STRANDED: 1 MALES: 1 FEMALES:
STORAGE PRIOR TO NECROPSY: ambient temperature overnight (winter)
DEGREE OF AUTOLYSIS: mild/ fresh (Code 2 Geraci score)
SIGNALMENT (species/ age/ sex): *Tursiops aduncus*/ 14yo/ male
WEIGHT (kg): 85.8 kilograms
LENGTH (cm): 195cm

HISTORY: animal had been observed losing weight for several weeks. Identical pattern to other ADS inner dolphins – rapid emaciation and death. Animal observed deep diving for approximately 1 week prior to death. Suspect foraging in muds on sea floor.

SUMMARY (DDx from gross exam):

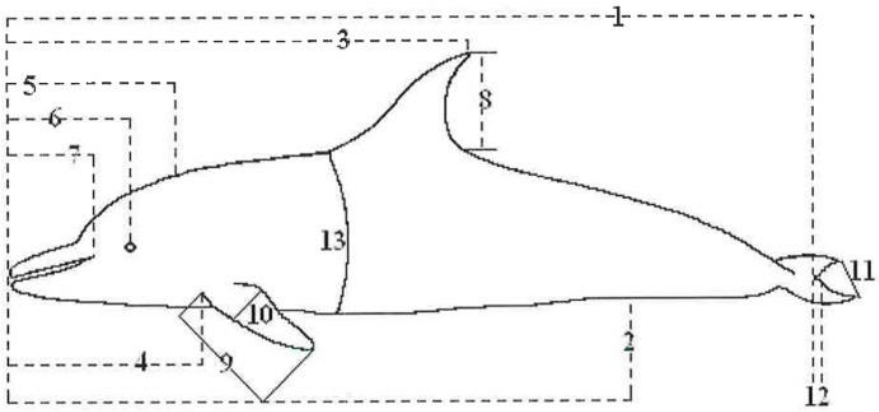
The major findings at gross post mortem were emaciation, multifocal skin lesions consistent with dermal abscesses, a focal small pulmonary abscess, and most significantly, examination of cerebrospinal fluid suggest inflammation (meningitis, meningoencephalitis). Culture of CSF and heart blood were negative, toxoplasma serology and CeMV testing pending. Histopathological examination of the brain is pending.

The oral cavity showed is significant irregular dental wear and gingival recession and resorption. Small spleen and testes size noted (please see report for details). Examination of other organs was grossly unremarkable. Skin lesion analysis pending histopathology. Interpretations in the brain and other organs pending histopathological analysis. Culture results provided within report.

Registered Specialist Veterinary Pathologist

Morphometric Measurements

1. Total length (tip of upper jaw to deepest part of fluke notch): 195cm	9. Length of flipper (anterior to tip): 37cm
2. Tip of upper jaw to centre of anus: 138cm	10. Width of flipper (maximum): 15.5cm
3. Tip of upper jaw to tip of dorsal fin: 113cm	11. Width of tail flukes: 52cm
4. Tip of upper jaw to anterior insertion of flipper:46cm	12. Depth of notch between flukes: 50mm
5. Tip of upper jaw to blowhole: 27cm	13: Maximum girth (+ distance from upper jaw): 100cm (78cm)
6. Tip of upper jaw to centre of eye: 27cm	14. Girth at anus: 64.5cm
7. Length of gape: 23cm	15. Girth at eye: 80cm
8. Height of dorsal fin (tip to base): 20cm	16. Girth at front of pectoral fin ; 89.4cm



Condition of Animal and Skin

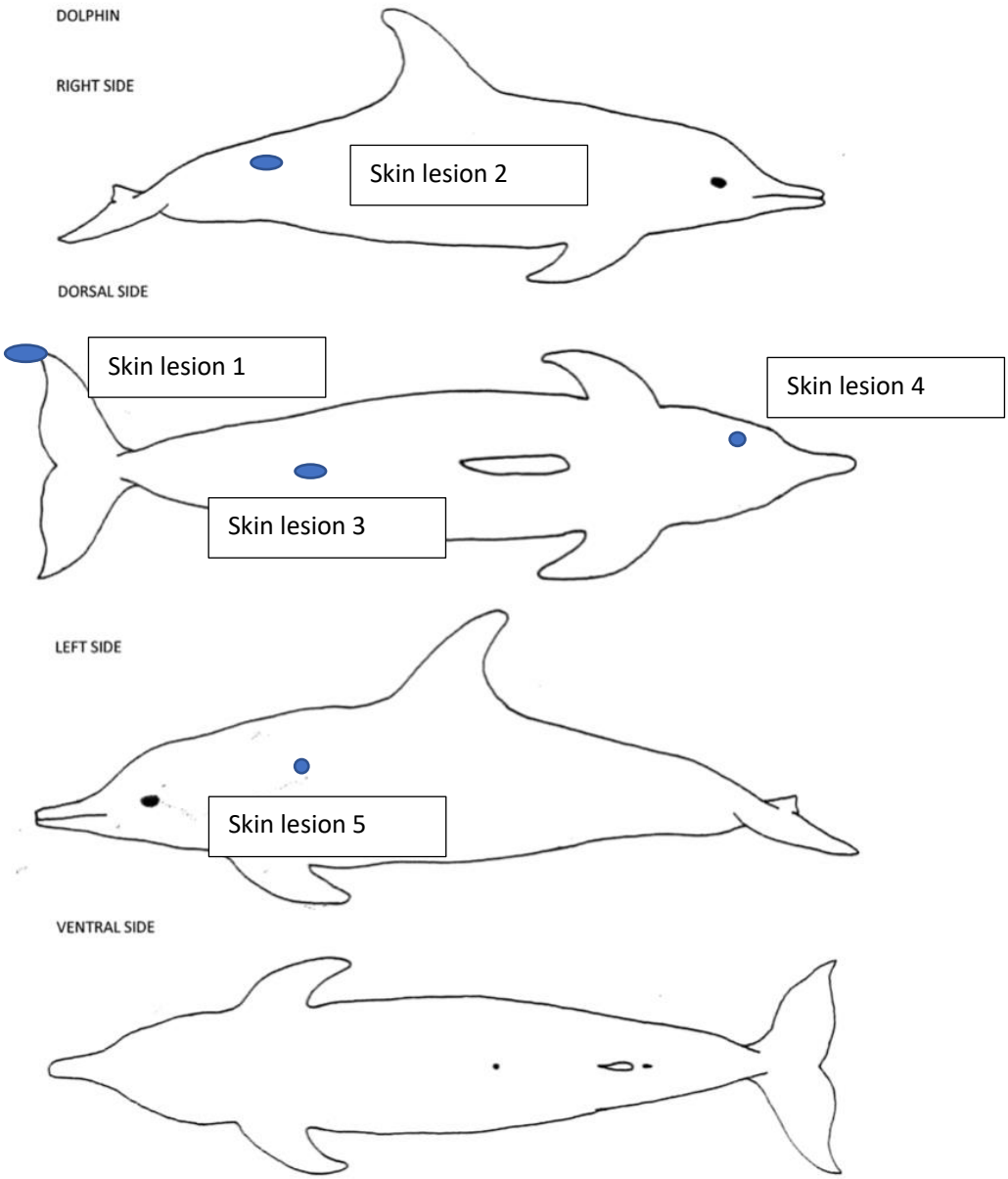
Body Condition: <u>Moderately/ very emaciated</u>

Presence or absence of:

1. Dorsal concavity behind the head - 2
2. Concavity along the lateral body - 2
3. Convexities along the lateral peduncle - 2
4. Convexities in the thorax - 2

Rate absent (0), 1 (visible but not extreme condition), 2 (clearly visible/extreme condition)

Skin and Subcutis Condition (colour/ condition):



Skin lesion 1: At the left lateral edge of the peduncle there is a locally extensive linear region of epidermal erosion and ulceration, with exposure of the underlying irregularly thickened and erythematous dermis.
Microbiology: Heavy mixed growth of *Acinetobacter lwoffii* and *Pantoea agglomerans*

Skin lesion 2 is a ~14mm x 5mm irregular full thickness epidermal defect which exposes the underlying dermis. On sectioning the local underlying superficial dermis is mottled red and yellow and softened (interpreted as inflammation, dermal abscess)

Microbiology: no growth.

Skin lesion 3 is a ~12mm x 5mm irregular full thickness epidermal defect which exposes the underlying dermis. On sectioning the local underlying superficial dermis is mottled red and yellow and softened (interpreted as inflammation, dermal abscess)
Microbiology: moderate growth of *Vibrio gigantis*

Skin lesion 4 is a well demarcated ellipse of epidermal hypopigmentation. On sectioning the local underlying superficial dermis is mottled red and yellow and softened (interpreted as inflammation, dermal abscess)
Microbiology: no growth after 48 hours.

Skin lesion 5 is an elliptical hyperpigmented smooth edged depressed crater in the epidermis exposing the underlying dermis. On sectioning, a 2-3mm well demarcated region of the superficial dermis is softened, and mottled red and yellow (interpreted as inflammation, dermal abscess)
Microbiology: no growth after 48 hours.

Blowhole
No abnormalities detected. Airways clear and no foreign material noted.

Mouth **(tongue, teeth condition, lesions, mucous membranes)**

Left Upper: 22 (8-13 extensive severe wear of teeth)	**Right Upper:** 22 (18-20. Significant wear of teeth, and exposure and loss of alveolar bone)
Left Lower: 20	**Right Lower:** 20

Eyes **(discharge/ colour)**
No abnormalities detected.

Ears
No gross abnormalities detected. Left and right inner ears collected as per Morell protocol.

Genital Slit/ Anus
No gross abnormalities detected.

Body Cavities

No increase in or abnormal appearance of peritoneal or thoracic cavity fluid noted.

Subcutaneous Condition

No subcutaneous haemorrhage or oedema noted.

Gastrointestinal Tract

- ☐ Collect stomach and contents in large plastic bag for analysis at SAM

On incision of the soft tissues at the base of soft palette, soft thick yellow material oozed from the soft tissues. The source was unable to be identified. A swab of the lesion grew a moderate mixed growth of *Edwardsiella tarda* and *Vibrio alginolyticus*.

Oesophagus: NAD
Stomach (contents, ulcers, mucosa, parasites): not examined.
Intestine: Moderate intestinal fill. NAD
Peritoneum, mesentery, omentum: NAD

Liver

Weight: 1.56 kg (285mm x 210mm at maximum dimensions). **No parasites seen grossly Gall bladder/ Bile Duct/ Pancreaticoduodental duct: NAD**

Pancreas

- ☐ NAD

Respiratory System

Within the mid dorsolateral lung fields is a focal subpleural/ parenchymal abscess. No parasites seen grossly. Lung abscess microbiology: Heavy mixed growth of Edwardsiella tarda and Vibrio alginolyticus

Circulatory System

Heart blood collected and stored whole as well as spun and separated Heart weight: **440grams.** No gross abnormalities noted. Microbiology of heart blood revealed no growth.

Lymphatic System

Spleen: Weight: 20.3 grams (L39mm, W33mm). Spleen is firm, mottled white and dark red-purple, and multifocal subcapsular-capsular white plaques up to 5mm in diameter.

Thymus: Not identified
Scapular lymph node: ~10 x 5mm, mild enlargement
Mesenteric lymph node: Firm, prominent.

Musculoskeletal System

Blubber thickness (cm)	Mid-dorsal (between head and dorsal fin)	19mm
	Mid-ventral (just in front of umbilicus)	17mm
	Lateral (right)	15mm
	Lateral (left)	NR

Generalised muscle wasting consistent with inanition.

Urinary System

Kidneys:

Right L 158mm W78mm Weight: 250gm	**Left** L170mm W75mm Weight: 250gm

Bladder: No abnormalities detected. Urine collected and stored frozen.

Reproductive System

Right testis Weight: 66.9gm Length:190mm Height: 21mm Width: 35mm	**Left testis** Weight: 56.8gm Length: 145mm Height: 23mm Width: 40mm

Penis: No significant findings

Endocrine Glands

Thyroid/ Parathyroid: Total weight: 14.4grams (W60mm, W 30mm)**;** Parathyroids prominent, 7mm x 10mm and 10 x 5mm, firm, white

Adrenal glands:

Right Weight: 4.3grams L51mm W19mm	**Left** Weight: 4.3grams L35m W17mm

Pituitary: No significant findings

Brain: no significant gross findings

Spinal Cord: cervical region examined only, no significant gross findings.

Vertebral Column: NAD (skeletal preparation pending)

Bone Marrow (colour/ consistency): Not examined

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

CSF Cytology Report

Specimen Type: Cadaver
Macroscopic appearance: Slightly turbid & Slight red tinge

Biochemical Analysis:

CSF Glucose	0.1	mmol/L	Reference Interval 60-80% plasma or serum glucose
CSF Protein	3.03 H	g/L	Reference Interval <0.30g/L

Cell Count:

Nucleated cell count	182.0 H	cells/uL	Reference Interval <5 cells/uL

There were large numbers of ruptured cells and bare nuclei on the concentrated cytospin preparation, though there was still a significant population of in-tact leukocytes. A 200 cell differential count was performed and showed 95% lymphocytes, 3% large mononuclear cells/ macrophages, and 2% non-degenerate neutrophils. Lymphocytes were small to intermediate in size, the nuclei are round to slightly irregular in shape, and often contained moderate numbers of fine to chunky and slightly irregular purple cytoplasmic granules. Rare plasma cells were also noted. There were moderate amounts of blood and scattered crystalline material (likely contaminants from the collection tube). No convincing micro-organisms were seen.

Interpretation:
Lymphocytic pleocytosis

Comment:
Though the differential count may be somewhat inaccurate given the poorly preserved nature of many of the cells, overall cellularity and protein content of the CSF appears increased, and the majority of the in-tact cells are small to intermediate lymphocytes. General considerations for a lymphocytic pleocytosis include viral, protozoal (e.g. toxoplasma), and fungal infection. Immune-mediated disease or underlying neoplasia could also be considered. The granules seen in the lymphocytes are somewhat unusual (the granules are sometimes large and irregular in shape), though they are predominantly small to intermediate in size and otherwise do not show striking features of atypia.

Bacterial culture of the CSF revealed no growth.

Gross Morphological Diagnoses:

1. Body as a whole: Emaciation
2. Central nervous system: Meningitis – lymphocytic pleocytosis
3. Skin and subcutis: Multifocal dermal abscesses
4. Spleen: query atrophy vs hypoplasia
5. Testis: query atrophy vs hypoplasia
6. Lung: focal pulmonary abscess
7. Dental arcades: irregular dental wear with resorption (periodontal disease)

Comments:

The major findings at gross post mortem were emaciation, multifocal skin lesions consistent with dermal abscesses, a focal small pulmonary abscess, and most significantly, examination of cerebrospinal fluid suggest inflammation (meningitis, meningoencephalitis). Culture of CSF and heart blood were negative, toxoplasma serology and CeMV testing pending. Histopathological examination of the brain is pending.

There is significant irregular dental wear and gingival recession and resorption. Small spleen and testes size noted (please see report for details). Examination of other organs was grossly unremarkable. Skin lesion analysis pending histopathology. Interpretations in the brain and other organs pending histopathological analysis. Culture results provided within report.

Cadaver held frozen for transfer to SAM, Bolivar, only with stomach contents and organ samples.

A further report will be issued following availability of outstanding test results.

Microscopic Descriptions

Representative tissues are examined across sections 22-01721_1-20

1-4, Brain and cervical spinal cord: sections through the frontal cortex, temporal cortex pons, medulla and spinal cord are examined. There is mild infrequent perivascular mononuclear cell infiltration within the meninges, which occasionally extends into the leptomeninges. Multifocally scattered through the brain are rare mild perivascular mononuclear cuffs. There is mild vascular congestion and mild to focally moderate focal perivascular haemorrhage within the meninges.

5-6, Lung: there is a focal abscess characterised by a core of ghost- cellular outlines degenerate leukocytes, alveolar septae and cartilage, consistent with coagulative necrosis surrounded by degenerate and non-degenerate neutrophils, macrophages, lymphocytes, plasma cells and fibroblasts. Admixed with inflammatory cells are mixed bacterial colonies. Fibroblasts are arranged with collagen forming a thick capsule. Inflammatory infiltrates also show scattered multinucleated giant cells in the surrounding parenchyma, and there is a moderate increase and alveolar septal cellularity due to infiltration of small and medium-sized lymphocytes with lesser neutrophils and macrophages. In other sections of lung, parenchyma is very well preserved. Bronchiolar mucosa shows occasional segmental subepithelial mineralisation and rare multinucleated giant cells. ZN stain does not reveal mycobacterial agents.

7, Spleen: there is mild lymphoid hyperplasia and moderate central follicular lymphocytolysis.
Kidneys: no significant findings

8,Thyroid gland: no significant findings, however detailed morphometric analysis comparative study is pending.

Liver: there are rare scattered pigment laden granulomas. There is rare to mild multifocal infiltration of mononuclear cells within periportal regions no evidence of inflammatory and necrotising processes or degenerative processes are seen in the sections examined
9 adrenal gland: no significant findings. Detailed morphometric analysis pending - query mild nodular cortical hyperplasia

Liver, as describe above

10, heart: no significant findings

11: There is a mild increase in interstitial fibroplasia (fibrocollagenous interstitium surrounding seminiferous tubules 2- 3 layers thick, some variation). There is single to rarely bilayer laying of immature spermatogonia within tubules and mature stages (spermatids) are not noted, with differentiation across all maturation stages of spermatogonia not seen. Seminferous tubular epithelium ranges in thickness between 18 and 28 um and tubular diameter 60-75um in diameter (immature, Kemper et al J Mammology 95:2 2014). There are rare mild mononuclear inflammatory infiltrates throughout the testes. More detailed comparative morphological study of seminiferous tubule diameter, seminiferous epithelium, and interstitium is pending.

12, mesenteric lymph node: there is moderate central follicular lymphocytolysisis.
Testis: as above

13, proximal intestine, duodenum: no significant findings. Sections show mild to moderate autolytic change

14, mid intestine: no significant findings. Sections show mild to moderate autolytic change

15, distal intestine: sections show mild to moderate autolytic change. There is no significant findings, subtle changes may have been obscured by autolytic change

17 - Skin lesion 1 (lateral fluke): there is locally extensive loss of the epidermis and moderate to marked infiltration of the underlying ulcerated surface and dermis by degenerate and non-degenerate neutrophils, fibrin and hameorrhage. There is moderate spongiosis of the adjacent epidermis with and hyperplasia of the adjacent stratum externum

16 - Skin lesion 2: in the centre of the section there is focal epidermal loss (uilceration), and in the exposed dermis there is a moderate mixed perivascular to interstitial infiltrate of neutrophils, lymphocytes, lesser macrophages, and plasma cells. Covering the exposed dermis is a coagulum of fibrin embedded with degenerate leukocytes and superficial bacterial colonies. There is a moderate increase in dermal and subcutaneous collagen underlying the wound, with interstitial proliferation of fibroblasts and infiltration of mononuclear inflammatory cells. The adjacent epidermis shows moderate spongiosis and protein exudation with fusion of rete pegs

18– skin lesion 3, dorsal peduncle: as described for skin lesion 2

19 – skin lesion 4: there is focal epidermal ulceration and loss and moderately well demarcated infiltration of the underlying dermis by macrophages, degenerate and non-degenerate neutrophils, lymphocytes and palsma cells, frequently forming multifocal to coalescing pyogranulomas. The

centre of the lesion is characterised by necrotic debris and degenerate leukocytes. Gram and PAS stains are unremarkable. ZN stain reveals moderate numbers of acid-fast filamentous bacilli within inflammatory infiltrates.

20 – skin lesion 5, left lateral thorax: as describe for skin lesions 2 and 3

Microscopic Diagnoses

Skin lesion 1, lateral fluke: locally extensive ulcerative and necrosuppurative dermiatitis with intralesional bacteria (Microbiology: Heavy mixed growth of *Acinetobacter lwoffii* and *Pantoea agglomerans*)

Skin lesion 2, lateral tail peduncle: ulcerative and hyperplastic neutrophilic dermatitis with dermal fibrosis (dermal abscess, ruptured), (microbiology: no growth)

Skin lesion 3, dorsal tail peduncle: ulcerative and hyperplastic neutrophilic dermatitis with dermal fibrosis (dermal abscess, ruptured), (microbiology: moderate growth of *Vibrio gigantis*)

Skin lesion 4, left lateral dorsal head: subacute pyogranulomatous dermatitis with dermal rupture and intralesional acid fast bacilli (presumptive dermal mycobacterial abscess/ granuloma); microbiology: no growth on routine culture, mycobacterial culture pending

Skin lesion 5, left lateral thorax: ulcerative and hyperplastic neutrophilic dermatitis with dermal fibrosis (dermal abscess, ruptured); microbiology: no growth

Spleen and mesenteric lymph node: acute lymphocytolysis

Brain: mild nonsuppurative meningoencephalitis

Testes: immaturity

Lung: focal necrospuurative abscess with intralesional bacteria; microbiology: Heavy mixed growth of Edwardsiella tarda and Vibrio alginolyticus

Comment
Examination of the brain showed mild mononuclear infiltrates within the meninges and occasionally or rarely within the brain consistent with mild nonsuppurative meningoencephalitis. This finding is corroborative with the mild lymphocytic pleocytosis seen on cerebrospinal fluid analysis. These changes are aetiologically non-specific and further testing for viral and protozoal

agents such toxoplasmosis is required. Culture of the cerebrospinal fluid was negative and no aetiological agents were seen on cytological examination of the cerebrospinal fluid.

The skin lesion on the head is granulomatous dermatitis due to Mycobacteria. The identity of the species is pending further testing. A number of mycobacterial species may be present in marine environments, and are usually opportunistic pathogens in compromised individuals. Some Mycobacterial species may be zoonotic, and further advice is pending species identification.
The other skin lesions show a range of changes suspected to be both traumatic/abrasive such as the lateral tail peduncle, but dermal abscesses that have erupted through the skin at various stages of chronicity. Mixed bacterial organisms were isolated from these lesions, although some lesion showed no growth (such as the lesion with intralesional Mycobacteria – often slow growing or require specialised culture conditions). No evidence of the invasive ciliated protozoal organisms which have been seen previously in other dolphins were seen in these sections.

Lung lesion was a bacterial abscess – involvement of Mycobacteria in this lesion is not excluded. Organisms were not seen on ZN stain, however will be further examined by molecular techniques. Acute lymphocytolysis seen in the spleen and lymph nodes is consistent with acute stress preceeding death, but can also been seen in acute viral infections.

Histopathological examination of the gastrointestinal tract, liver, kidneys, adrenal glands and heart was largely unremarkable. Further morphological assessment of the thyroid gland and comparison with non ADS dolphins is pending. The testes were immature with scant mature spermatids seen; the morphology of the testes will be more closely examined through an objective Morphometric study comparing these testes with those of other dolphins both inside and outside the ADS.

Outstanding tests are toxoplasmosis serology and CeMV testing.

A per other investigations, a definitive cause of death was not identified, rather multiple chronic disease processes linked to opportunities organisms. Detailed examination of the brain for chronic neurodegenerative processes with a neuropathologist is underway in the coming weeks. Inner ears are fixed and held pending consultation with international experts in detection of acoustic damage in cetaceans.

Registered Specialist Veterinary Path