Veterinary Diagnostic Laboratory
School of Animal & Veterinary Science

CONFIDENTIAL AND PRIVILEGED INFORMATION

University of Adelaide Pathology reference: 21-02676
Owner name: DEW
Referring veterinarian or authorised agent: Third party details redacted for the purpose of release of this report into the public domain
Signalment (species/ breed/age/ sex): Tursiops aduncus (Indo-Pacific Bottlenose dolphin)
Time and date of death: 10am, 22/10/21, by barbiturate overdose
Time and date of post mortem examination: 1pm, 22/10/21
Carcase condition: fresh
Persons present: Post mortem examinations performed by Third party details redacted for the purpose of release of this report into the public domain

History:
Adelaide Dolphin Sanctuary resident juvenile male dolphin that has shown a sharp decline in condition in the previous 5 weeks. Euthanased on welfare grounds for post mortem investigation into ongoing morbidity and mortality in ADS dolphins. Assigned SAM accession number 21.038

MACROSCOPIC EXAMINATIONS:
External examination, body condition, skin and subcutis
A juvenile male dolphin known to be six years of age is received for post mortem examination. The cadaver weighs 53.8 kilograms, and is in thin to emaciated body condition (score 1), characterised by concavity ventrolateral to the dorsal fin and wasting of the epaxial muscles, depression posterior to the blow hole, and narrowing of the trunk with variable visibility of the ribs.

Body measurements are presented below (pending completion):
Total length: 165cm
Snout to anus: 1052mm
Snout to genital slit: 895mm
Snout to tip of dorsal fin: 985mm
Snout to origin of flipper: 370mm
Snout to blow hole: 165mm
Snout to eye: 180mm
Snout to ear:265mm
Snout to corner of mouth: 143mm
Eye to corner of mouth: 60m
Eye to blowhole (125mm curved), 130mm straight
Projection of mandible: 55mm
Snout length: 35mm
Length of flipper:315mm
Width of flipper: 124mm
Height of dorsal fin: 170mm
Length of base of dorsal fin: 275mm

There is notable shortening of the maxilla (brachygnathia superior) and upwards curvature of the mandible.
Multiple ulcerated cutaneous lesions are noted over the external surfaces (largest characterised below), along with multifocal firm raised white circumscribed cutaneous lesions over the pectoral flippers interpreted as scars.
Lesion 1 is located lateral to and at the caudal aspect of the dorsal fin and is characterised by a 10-15mm mm focal ulcerated red-yellow cutaneous mass rimmed by hyperplastic epidermis. On sectioning, red-brown discolouration (hyperaemia, inflammation) extends into the blubber.
Lesion 2 is an irregular cutaneous ulcerated mass 10mm x 20mm located at the dorsal lateral aspect midway along the right side of the tail. A central mottle red crater is rimmed by a hyperplastic epidermis, with a core of red-orange purulent material which extends into the blubber.

Smaller and similar lesions with less pronounced epidermal thickening are also located on the right distal tail peduncle above, and the ventral intermandibular skin, as well as over the dorsal surface of the fluke.

Serous cavities
No increased fluids are noted on opening of the thoracic and abdominal cavities, and organ positioning is normal.

Alimentary system
The oesophagus contains a single small shrimp. The main stomach is largely empty aside from brown mucous and crustacean shells. The forestomach contains approximately 250mls of watery digesta that contains a few shrimps. Some shrimps are orange and have the appearance of being cooked. All stomach contents collected for analysis at the South Australian Museum. There are two raised crater like ulcers, surrounded by a rim of hyperplastic squamous mucosa.

The pyloric stomach contains approximately 50mls of orange-brown translucent fluid (blood tinged). The mucosal surface is glistening, orange, and has multifocal to coalescing bright red pin point to 10mm diameter irregular non-raised bright red discolorations (petechial and ecchymotic haemorrhages.

The mesenteric lymph nodes are moderately to markedly enlarged and firm on sectioning (interpreted as reactive hyperplasia, +/- inflammation and fibrosis). The intestines contain scant green to brown mucoid digesta. The mucosa is hyperaemic and mildly thickened, more notably in the distal third. The distal intestine and rectum is largely devoid of contents with a small volume (<2mls) of dark brown liquid faecal material able to be collected.

The liver weighs 1.26kilograms and is dark mahogany to brown. The capsular surface is mildly irregular and undulating and the parenchyma is irregularly firm on palpation and on sectioning (interpreted as congestion, hepatic fibrosis, further characterisation pending histopathology).

Respiratory system
The respiratory tract is largely grossly unremarkable. Rare subpleural cysts 2mm in diameter present over the pleural surfaces of the caudal and dorsal lung fields.

Cardiovascular system
Pericardium of apex, right viaticum was discoloured dark red (tissue sample has been collected.

Musculoskeletal system
The is moderate diffuse reduction in muscle mass of the body as a whole (consistent with inanition)

Lymphoreticular system
The spleen is congested and weighs 25 grams.

Urinary system
No significant findings (NSF). Urine collected for analysis

Reproductive system
NSF. Testes collected.

Endocrine system
The left adrenal gland weighs 2.2 grams and the right adrenal gland weighs 2.2grams. The thyroid gland weighs 8.4 grams.

Special senses
Left eye: there is a focal raised 3-5mm diameter firm white opacity arising from the central cornea, surrounded by a grey rim of decreased corneal opacity (chronic corneal ulcer). Both left and right eyes collected and will be further characterised by histopathology.

On sectioning through the external ear canal, a thick grey-brown exudate oozes from the canal. On dissection of the dorsal palate a similar exudate is also noted to ooze from the eustachian region. The left tympanic bulla was filled with a grey-brown exudate which was also visible through the cochlear window of the inner ear suggestive of a middle-ear and inner-ear infection. The petrous part of the temporal bone appeared rough and irregular suggestive to an inflammation of this area. The right ear was considered normal.

Wright giemsa stained cytology of the exudate from the left ear canal revealed a highly cellular preparation within an eosinophilic background, with good to excellent cell spread and staining and moderate cell preservation. The cells comprise a heterogenous population predominated by degenerate and non-degenerate neutrophils, macrophages, rare eosinophils, and sloughed epithelial cells. Admixed with inflammatory cells are numerous bacterial colonies, comprising cocci in chains, or less often short rods.

Nervous system
Brain is unremarkable grossly. Further dissection will occur after 7-10 formalin fixation

Veterinary Diagnostic Laboratory
School of Animal & Veterinary Science

THE UNIVERSITY of ADELAIDE

Antemortem blood examination

TURSIOPS SPP. PLASMA BIOCHEMISTRY

				REFERENCE INTERVAL (ZIMS)	
TEST	*UNITS*	Hunter (UofAVDL)	Doc (ZoosSA)	ALL COMBINED	MALE
Na	*mmol/L*	152	155	150-162	152-162
K	*mmol/L*	4.4	5.6	3.2-4.8	3.2-4.8
Chloride	*mmol/L*	107	111	113-128	112-131
Total CO2	*mmol/L*	30	21	NA	NA
Anion gap		19.5			
BUN	*mmol/L*	22.8	20.7	12.3-27.3	13.6-27.3
Creatinine	*umol/L*	69.7	58	44-165	44-166
Uric Acid	*umol/L*		16	18-67	NA
Total Protein	*g/L*	81.4	94.4	54-86	52-84
Albumin	*g/L*	40.9	46.4	NA	NA
Globulin	*g/L*	41	48	16-49	21-44
A:G ratio	*ratio*	1.00	0.967	0.7-3.0	NA
ALP	*U/L*	132	260	74-910	84-1662
AST	*U/L*	255	346	119-616	108-497
ALT	*U/L*	31.6	58	12.0 - 73.0	12.0 - 73.0
GGT	*U/L*	45.7	36	12.0 - 86.0	12.0 - 86.0
GLDH	*U/L*	1.7			
Cholinesterase	*U/L*		107	NA	NA
LDH	*U/L*		749	295-788	277-1299
Ca	*mmol/L*	2.28	2.47	2.0-2.7	2.0-2.7
Phos	*mmol/L*	1.8	2.3	1.13-2.34	1.16-2.49
Mg	*mmol/L*	1.05	0.76	NA	NA
Iron	*umol/L*	11.3	12.5	11.6-57.6	11.2-40.3
Cholesterol	*mmol/L*	3.6	3.69	2.09-8.49	1.98-8.88
Triglyceride	*mmol/L*	0.6	0.74	0.21-1.21	0.2-0.87
Glucose	*mmol/L*	5.6	6.3	3.22-7.94	3.88-8.38
Creatine kinase	*U/L*	267	248	37-271	29-309
Lipase	*U/L*	10			
Amylase	*U/L*	1			
Tbil	*umol/L*	3.8			
D-3 hydroxybutarate	*mmol/L*	0.02			
FRAP	*mmol/L*		0.71	NA	NA
TEAC	*mmol/L*		2.33	NA	NA
Ascorbic Acid	*umol/L*		32.7	NA	NA

Biochemistry results are largely unremarkable, consultation with Third party details redacted for the purp for his opinion pending.

Veterinary Diagnostic Laboratory
School of Animal & Veterinary Science

THE UNIVERSITY of ADELAIDE

TURSIOPS SPP. HAEMATOLOGY

				REFERENCE INTERVAL (ZIMS)	
TEST	*UNITS*	Hunter (UofAVDL)	Doc (ZoosSA)	ALL COMBINED	MALE
WBC	*10^9cells/L*	17.43	14.54	3.1-12.5	3.4-13.3
Lymphocyte (%)	*%*	6.30	24.60	3.0 - 32.0	3.0 - 32.0
Lymphocyte ABS		1.10	4.29		
Monocyte (%)	*%*	0.80	2.20	0.4-9.3	0.4-10
Monocyte ABS		0.14	0.38		
Neutrophils (%)	*%*	89.00	72.80	54.2-88	52.9-84
Neutrophil ABS		15.51	12.69		
Eosinophils (%)	*%*	3.50	0.40	0-25	0-26
Eosinophil ABS		0.61	0.07		
Basophils (%)	*%*	0.30	0.00	0-2	0-1.6
Basophil ABS		0.05	0.00		
Est WBC count	*10^9cells/L*		10	NA	NA
Manual Lymphocyte (%)	*%*	5	7	4.7-39	May-40
Manual Monocyte (%)	*%*	2	1	0-8.0	0-9.0
Manual Neutrophils (%)	*%*	90	92	45-87	43-87
Manual Eosinophils (%)	*%*	3	0	1-23.4	Jan-24
Manual Basophils (%)	*%*	0	0	0-1	0-1.1
Fibrinogen	*g/L*	4			
RBC	*10^12cells/L*	4.86	4.64	2.6-4.03	2.7-4.07
HGB	*g/L*	165	158	115-173	113-177
HCT	*L/L*	0.501	0.4	0.33-0.51	0.32-0.51
MCV	*fL*	103.1	88	104.8-147.5	102.5-152.6
MCH	*pg*	34	3.41	2.33-3.33	2.32-3.37
MCHC	*g/L*	330	387	303-412	301-417
RDW	*%*	14.7	17	11.3-32.8	11.2-25
Platelets	*10^9cells/L*	204	67	50-237	55-255
MPV	*fL*	14.1	10.7	3.6-17.0	3.6-17.1
Total solids		89			
PCV		50			
Film morphology		No toxic change, no left shift, RBC and WBC morphology normal			

Haematology shows a stress leukogram and haemoconcentration (dehydration). Blood film morphology normal.

Toxoplasma serology (IDVET): pending

Brucella serology (Rose Bengal Test): negative

Urinalysis
Pending

GROSS MORPHOLOGICAL DIAGNOSIS

1. *Body as a whole: emaciated body condition*
2. *Skin and subcutis: multifocal cutaneous ulcerations with subcutaneous abscessation*
3. *Left ear: necrosuppurative otitis media and interna with localised osteolysis (presumptive).*
4. *Pyloric stomach: haemorrhagic gastritis*
5. *Intestinal tract: segmental hyperaemia and mild thickening with moderate mesenteric lymphadenomegaly (reactive hyperplasia, inflammation, other)*
6. *Liver: mild parenchyma fibrosis (presumptive)*
7. *Skull: brachygnathia superior*
8. *Left eye: chronic focal corneal scarring*

COMMENT
The most significant findings identified at post mortem were thin to emaciated body condition, necrosuppurative otitis media and interna of the left ear (bacterial), multiple cutaneous abscesses, haemorrhagic gastritis of the pyloric stomach, and a enteropathy with moderate mesenteric lymph node enlargement.

A severe otitis media and interna was identified in the left ear tissues, associated with localised bone softening, discolouration and fragmentation, interpreted as osteomyelitis. Purulent exudate was identified oozing form the external ear canal, as well as from the Eustachian tubes on dissection of the pharyngeal region. Multiple nematodes were identified within the exudate, however cytology of exudate within the ear canals revealed an inflammatory exudate predominated by degenerate neutrophils with numerous bacterial colonies present, consistent with bacterial otitis media and likely interna – histopathology of the inner ear and cultures of the exudate are pending and will be issued as available.

The significance of the trematodes in the inner ear and sinuses is likely that they are symbiotic and of no/ little clinical significance, and are reported commonly in dolphins in the ADS and further afield. For consideration however, there are reports of fatal parasitosis associated with severe Nasitrema sp. pterygoid sinusitis and otitis media and otitis interna with brain invasion, with the severity of lesions in these described cases leading authors to believe lesions may have contributed to stranding and death in affected individuals (Diaz-Delgado et al 2018).

The severity of changes present in Hunter's inner ear are of severity such that there would be concerns for the long-term viability of this animal in regard to hunting, foraging and proprioception. Furthermore infection involving the bony tissues in this region would have been difficult to manage (protracted courses of intensive antibiotics) with a guarded prognosis. Changes in the gastrointestinal tract will be further characterised by histopathology, and interpreted in light of range of samples submitted for microbiological investigations (inclusive of enteric pathogens such as Salmonella, E. coli etc))

The shortening of the maxilla (brachygnathia superior) is a congenital deformity that has been present at birth. There is some upwards curvature of the rostral mandible in response to the lack of resistance above. This deformity has been reported in bottlenose dolphins previously (Post et al 2021 Aquatic Mammals Vol. 47, Issue 4). It is not thought to have interfered with feeding in this animal, as it is presumed Hunter would have presented at a much earlier stage in his life in poor body condition had this been the case.

A wide range of samples have been collected for histopathology, bacterial culture (see sites above) as well as preserved frozen for toxicological analysis in the near future. Serological tests for exposure to *Toxoplasma* and *Brucella* are pending.

A further report will be issued as outstanding test results become available. Culture and histopathology will allow improved interpretation of the significance of lesions, and the role that other factors such as immunosuppressive disease may play.

ADDENDUM (11/11/21)

Microbiology

Left ear (external canal and internal swabs): Heavy growth of Edwardsiella tarda

Heart blood: No growth, aerobic and anaerobic, at 48 hours

Pyloric stomach fluid: Light growth of Clostridium perfringens (typing pending)

Skin lesion 1 (tissue and swab collected antemortem): Heavy growth of Vibrio harveyi

Skin lesion 2 (tissue and swab collected antemortem): Moderate growth of Vibrio harveyi

Proximal intestine: No growth, aerobic or anaerobic, at 48 hours.

Distal intestine: Moderate growth of Edwardsiella tarda and Photobacterium damselae. Light growth of mixed anaerobes including Clostridium perfringens

Faeces: Heavy growth predominantly Edwardsiella tarda and Photobacterium damselae. Light growth mixed anaerobes including Clostridium perfringens.

Blow hole: Moderate growth of Vibrio alginolyticus and moderate growth of Salinivibrio proteolyticus

Anus: Moderate mixed growth predominantly Photobacterium damsela

Mouth: Moderate mixed growth (including Vibrio anguillarum)

Left eye: No growth, aerobic or anearobic, at 48 hours.

Right eye: Moderate mixed growth (including Pseudomonas sp and Actinobacter lwoffii

Genitals: Moderate mixed growth including Photobacterium damsela

MICROSCOPIC DESCRIPTIONS

Pituitary gland: No abnormalities detected (NAD)

Adrenal gland: NAD. Microscopic cortex to medulla ratio 1:1.1

Cervical (C1) spinal cord: NAD

Skin lesion 1: there is focally extensive epidermal loss (ulceration) and extensively replacement of the underlying dermis and blubber by densely cellular mixed inflammation comprising degenerate and non-degenerate neutrophils, macrophages, lymphocytes, plasma cells and fibroblasts with variable fibrin exudation, frequently forming coalescing pyogranulomas. Opportunistic ciliated protozoal organisms measuring up to 100um with a macronucleus and vacuolated cytoplasm are present within superficial inflammation as well as within deep blubber pyogranulomas. Ulcerated regions are covered by a thick coagulum of haemorrhage, fibrin, degenerate leukocytes and myriad bacteria. Gram stain reveals bacteria as Gram negative curved large rods consistent with *Vibrio* spp, visible predominantly in the more superficial aspects of skin lesions.

Thyroid gland: NAD – further characterisation of morphology with reference to archived samples pending.

Skin lesion 2: as described above for skin lesion 1, with extensive invasion by protozoal agents associated with necrosuppurative inflammation, including into the deep blubber layers. The rim of epidermis

surrounding the region of ulceration is hyperplastic and spongiotic, with exudation of fibrin and degenerate leukocytes.

Skin lesion ventral neck: There is focal epidermal ulceration and infiltration of the underlying dermis by degenerate and non-degenerate neutrophils, fibrin, lesser macrophages, lymphocytes and plasma cells. Admixed with inflammatory infiltrates are rare ciliated protozoal organisms. The lesions is similar to described for skin lesions 1 and 2, But more superficial and acute in nature.

Spleen: Mild congested. Scattered lymphoid follicles are observed, with approximately one third of observed follicles with developed germinal centres (reactive change, mild).

Prescapular lymph node: there is moderate follicular hyperplasia with formation of germinal centres. Medullary sinuses contain low to moderate numbers of small lymphocytes, macrophages, and low numbers of neutrophils and eosinophils.

Mesenteric lymph node: The node is characterised and identified by a thick fibrocollagenous and muscular capsule and prominent stroma which dissects between lymphoid tissue (normal anatomic variation). There is mild reactive hyperplasia with infrequent germinal follicles. Medullary and subcapsular sinuses frequently contain moderate numbers of eosinophils, admixed with small lymphocytes. Germinal follicles are occasionally depleted/ hypocellular.

Lung: Mild diffuse congestion. Bronchiolar epithelium is extensively lost, often attenuated, low columnar to cuboidal and rarely squamous, with occasional replacement by basophilic refractile amorphous material (mineral, other). Bronchiolar lumina and some alveoli occasional contain flocculant eosinophilic material suspended with low numbers of leukocytes.

Main stomach: NAD

Kidney: NAD

Forestomach: There is focal erosion and segmental ulceration of the squamous mucosa, with infiltration of moderate numbers of degenerate and non-degenerate neutrophils and fibrin exudation in the regions of exposed submucosa. No aetiological agents are observed.

Pancreas: subjectively, mild atrophic change of pancreatic aciniar cells.

Pyloric stomach: there is extensive superficial to deep coagulative necrosis of the lamina propria (DDx rapid autolysis) with multifocal tissue mineralisation, congestion, and multifocal haemorrhage. Rare large bacterial rods are seen invading into the necrotic mucosa. There are scattered lymphoid aggregates in the deep aspects of the mucosa (normal finding).

Intestines: There is mild autolytic change. Intestinal villi are shortened/ blunted, and there is superficial infiltration and expansion of villi by lymphocytes, macrophages, neutrophils and eosinophils, with multifocal lymphoid aggregates within the lamina propria.

Colon: extensive superficial mucosal autolysis with denuding of luminal epithelium. Denuded stroma is extensively lined by bacterial rods, some which show invasion into the mid lamina propria.

Pectoral skin: no significant findings

Liver: there is mild periportal fibrosis and moderate diffuse infiltration of periportal interstitium by moderate numbers of lymphocytes, plasma cells and low numbers of neutrophils (nonsuppurative to mixed periportal

hepatitis), with infrequent small inflammatory cell aggregates within the parenchyma. Hepatocellular cords are moderately and diffusely thinned (atrophic change). There is variable moderate sinusoidal congestion.

Heart: NAD

Skeletal muscle: NAD

Right and left tympano-periotic complexes – pending decalcification.

Brain: pending

MICROSCOPIC DIAGNOSES

1. Skin, multiple sites: multifocal chronic-active ulcerative and necrosuppurative dermatitis with intralesional Gram negative bacteria and invasive ciliate protozoa.
2. Lung: bronchiolar epithelial degeneration and mineralisation, with attenuation and reepithelialisation
3. Liver: moderate mixed chronic-active periportal hepatitis and mild periportal fibrosis
4. Pyloric stomach: locally extensive mucosal coagulative necrosis and haemorrhage with mineralisation and rare intralesional large bacterial rods
5. Intestine: mild to moderate diffuse mixed lymphohistiocytic, eosinophilic and rarely neutrophilic enteritis with villous blunting; variable luminal proliferation of large rod bacteria (distal intestine)
6. Left prescapular lymph node: reactive hyperplasia
7. Mesenteric lymph node: reactive hyperplasia with mild sinus histiocytosis and eosinophilia

COMMENT

The most significant findings in this dolphin were poor body condition, congenital malformation of the maxilla and a number of opportunistic infections (further detail is provided below), as well as a enteropathy and enteric microbiological cultures consistent with intestinal dysbiosis. The presence of these multiple infections and an enteric dysbiosis strongly suggests this animal was immunosuppressed or compromised, the cause of which may include viral disease and exposure to toxicants, xenobiotics and pollutants – test results pending.

Skin lesions are characterised by an ulcerative and necrosuppurative dermatitis – the spectrum of lesions seen reflects varying stages of chronicity and progression. All skin lesions were associated with invasive protozoal agents and bacteria, and *Vibrio harveyii* was cultured in near pure growth from skin lesions with bacteria visible histologically in the more superficial aspects of lesions. Protozoal agents seen in the skin lesions are morphologically consistent with those described previously by Schulmann et al 1999, and have been observed as opportunistic causes of dermatitis in ADS dolphins previously (Star). These organisms are described as commensal organisms seen in up to 50% of bottle nosed dolphins (e.g. in blow hole) in the northern hemisphere, and are thought to be opportunistic invaders of pre-existing ulcers and skin lesions. The high incidence of dermatitis with invasive ciliates in the Atlantic bottlenose dolphin morbillivirus epizootic was thought to be due to the immunosuppressive effect of morbillivirus and resulting secondary infections, and not a direct effect of viral infection of the skin. Morbillivirus testing has not yet been performed for this individual, but previous animals have tested negative on the basis of testing of lung tissue. More extensive testing of other tissues for chronic CeMV infection and immunosuppression is underway.

Skin lesions are likely to be indicators or poor underlying health and immunosuppression, and infectious agents detected in association with skin lesions are opportunistic invaders from the marine environment.

Skin lesions are not considered the cause of the animals decline, rather a proxy for poor health due to other aetiology. No evidence of mycobacterial infection (either through culture or through microscopy and special staining) was seen.

The spleen was morphologically unremarkable but was very small, weighing 25grams only. Reported spleen weights in the Northern hemisphere bottlenose dolphins in a study by Cowan et al (1999) reported spleen weight minimum as 75grams, seen in a sexually immature animals weighing 45kilograms. Whilst lymphoid germinal follicles were observed in Hunter's spleen, there are concerns for an underdeveloped or atrophic spleen in this individual. Immunohistochemical studies will be performed on Hunter's spleen, along side lymphoid tissues from other dolphins from the ADS and outside the ADS to more closely assess follicle development and lymphocyte subsets. Further assessment of the thyroid, as well as serum thyroid hormones, is pending.

The most striking change in the lung is the degeneration, loss and mineralisation of bronchiolar epithelium - this changes has been observed previously in ADS dolphins and partially attributed to autolysis, however given the fresh nature of the tissues in this case, this change is considered significant. Virological testing is underway (Morbillivirus, calicivirus, influenza A and herpesvirus).

The pyloric stomach showed acute to subacute mucosal necrosis, haemorrhage and mineralisation microscopically, changes which are typical for early stage gastric ulcers that may be due a variety of causes, including chronic stress, systemic metabolic disturbances, vascular compromise to stomach wall, and infectious agents.

Occasional large rods consistent with Clostridia were seen invading the necrotic pyloric stomach mucosa, and *Clostridium perfringens* was isolated by culture from pyloric stomach, as well as other segments of the intestinal tract. Given the fresh nature of the tissues, it is unlikely that bacteria observed in tissue are post mortem invaders, and although *Clostridium* spp are normal inhabitants of the gastrointestinal tract, they may also be associated with diarrhoea and/ or necrohaemorrhagic enteritis when in high numbers and associated with toxin production. A definitive diagnosis of Clostridial disease is therefore usually rendered with the findings of a necrotizing enteritis with numerous Gram-positive bacilli present in the inflammation during histopathological examination of the affected tissues. Clostridial isolates within the intestinal tract may be significant in this animal in light of the histological changes seen. Typing of the Clostridial species is pending.

Clostridium perfringens type A infections have been associated with two cases of acute necrotizing enteritis cases in captive bottlenose juvenile dolphins (Salbany et al 2011), with clinical signs of acute onset, anorexia, fast swimming and a high respiration rate. Clostridial enteropathies in domestic species are often associated with intestinal dysbiosis, with a range of observed disease from diarrhoea to necrohaemorrhagic enterocolitis. A recent review of microbiota in captive cetaceans of poor health condition found that gut microbial communities in captive common bottlenose dolphins were dominated by a number of pathogenic bacteria, including *Clostridium perfringens*, *Vibrio fluvialis*, and *Morganella morganii*, reflecting the poor health condition of these animals (Bai et al 2021). Given the isolation of other pathogenic bacteria from the gastrointestinal tract of Hunter in heavy growth, such as *Edwardsiella* and *Photobacterium*, it is highly likely he was suffering from a significant gastrointestinal dysbiosis, with overgrowth of normal bacteria by pathogenic and opportunistic organism.

Edwardsiella tarda was isolated from the left ear and from multiple intestinal sites. *Edwardsiella* species are common marine inhabitants, and are a known cause of septicemia in fish. Reports in cetaceans vary between opportunistic infections and more severe disease such as necrotizing enterocolitis and/or septicemia, which can appear similar to salmonellosis. Animals that develop infections with *Edwardsiella*, as in this case, are usually debilitated or stressed, and ingestion of contaminated fish is a likely source of infection.

Diffusely along the length of the intestine there was mild mixed inflammation, with concerns for villous blunting and attenuation. This may reflect dysbiosis and overgrowth of pathogenic bacteria, or reflect diet perturbations (see comments below regarding maxilla). The gastrointestinal tract was largely empty, with scant faecal material present. No parasites were seen grossly, although not excluded on this basis. Too scant a faecal sample was present and no faecal parasitology was performed. Given the presence of eosinophils in the intestinal mucosa, enteric parasitism should be considered likely. Whilst endoparasites are considered a normal finding in wild dolphins, imbalances in parasite numbers may be seen with compromised health and contribute to overall poor health. Liver inflammation is centred in the peripotal region (mixed periportal hepatitis), and this form of liver inflammation is typical for reflecting portal drainage of inflammation from the gastrointestinal tract and/ or from the systemic circulation.

The animal was seronegative for Brucella exposure. Toxoplasma serology is pending.

Whilst the shortened maxilla was initially considered to have lesser influence in the decline of this animal, it is possible that this malformation and its subsequent effect on function led to abnormal feeding and hunting behaviours in Hunter. Therefore he may have been targeting atypical prey species and therefore been more prone to enteric infection or malnutrition. The report on gastrointestinal analysis provided by SAM is present as Appendix 1 at the bottom of this report.

An important focus of investigation now is the effect of toxicants/ xenobiotics and pollutants in tissues from these dolphins, and determination of any role that these factors may play in the compromise and immune suppression of these animals. Testing for systemic accumulation of toxicants is currently underway, assisted by the EPA and environmental toxicologists from CSIRO. There are numerous and well recognised reports from worldwide discussing the effect of toxicants and pollutants on declines marine mammal health and increasing susceptibility to infectious disease; the challenge in this investigation will be in elucidating which factors (toxicants, environmental stressors, others) are contributing to increased susceptibility to infectious disease and declining health in the ADS dolphin population.

A further report will be issued as outstanding test results become available.

Pending tests:

1. Toxoplasma serology
2. Histopathology of left and right ear/ tympano-periotic complexes (pending decalcification)
3. Histopathology of the brain
4. Toxicant/ xenobiotic testing of tissues
5. Endocrine testing (blood, sera)
6. Viral testin (CeMV, Influenza A, herpesvirus)

Third party details redacted for the purpose of release of this report into the public domain

REFERENCES

Schulmann FY, Lipscomb TP. Dermatitis with invasive ciliated protozoa in dolphins that died during the 1987-1988 Atlantic bottlenose dolphin morbilliviral epizootic. Vet Pathol. 1999;36:171–4.

Salbany, Bernal, Bossart, Wernery, Thomas, Raghaven and Syriac: Clostridium perfringens Alpha Toxin Infection in a Group of Indo-Pacific Bottlenose Dolphins (Tursiops aduncus) in the United Arab Emirates (UAE) Proceedings of IAAAM, 2011

Bai S, Zhang P, Zhang C, Du J, Du X, Zhu C, Liu J, Xie P and Li S (2021) Comparative Study of the Gut Microbiota Among Four Different Marine Mammals in an Aquarium. Front. Microbiol. 12:769012. doi: 10.3389/fmicb.2021.769012

Toxicology of Marine Mammals (Vol. 3) Joseph G. Vos, Gregory D. Bossart, Michel Fournier, Tom O'Shea (eds), 2003, Taylor and Francis, London, UK

APPENDIX 1

To: Third party details redacted for the purpose of release of this report into the public domain

Re: Prawn identifications

Examined 27/10/2021 & 2/11/2021 by Third party details redacted for the purpose of release of this report into the public domain

Stomach contents included 6 fragments of prawn, which we labelled A-F.

Found in oesophagus

A. Thorax and tail of prawn ~ 5.2 cm in length. Striped appearance, speckled. ***Metapenaeus bennettae* Racek & Dall, 1965**

Found in stomach

B. Head only of a prawn ~ 3.2 cm in length. ***Metapenaeus bennettae* Racek & Dall, 1965**

C. Thorax only of a prawn ~ 3.4 cm in length (8 mm thick not including legs). Speckled (similar to A). ***Metapenaeus bennettae* Racek & Dall, 1965**

D. Head only – no distinguishable features, soft translucent carapace with no flesh left. Not able to be identified.

E. Partially digested whole body of carid shrimp – Same as 'F'. (F is a more complete specimen.) ***Alpheus novaezelandiae*** Miers, 1876

F. Partially digested whole body of carid shrimp – Same as specimen 'E'. ***Alpheus novaezelandiae*** Miers, 1876

Note on distribution

M. bennettae (greentail prawn) is a sub-tropical prawn, found mainly on the east coast, but it has previously been reported that there is a small population in GSV and Port river (pers. Comm, by PIRSA on a previous ID query).

A. novaezelandiae (New Zealand pistol prawn) is found all around Australia as well as New Zealand.