BRITISH COLUMBIA
The Best Place on Earth

Ministry of Agriculture

Animal Health Centre

AAVLD - Accredited Laboratory

Ministry of Agriculture
1767 Angus Campbell Road
Abbotsford BC V3G 2M3
Telephone : (604) 556-3003
Facsimile: (604) 556-3010
Toll-Free : 1-800-661-9903

Final Report AHC Case: 16-1760

Last Updated: 09/29/16 2:39 PM
Pathologist: Stephen Raverty, DVM
Received Date: 04/04/16
Collected Date: 04/04/16
Client Ref No: L95

Veterinarian: **Dr. Marty Haulena**
Clinic: **Vancouver Aquarium**
Phone: (604) 659-3468
Fax: (604) 659-3469

Submitter: **John Ford**
Phone:
Fax:
Owner: **Pacific Bio Station J. Fo**
Phone:
Fax:(250) 756-7053

Animal Data
Species: Killer Whale
Breed:
Sex: M
Age: Adult
Premise ID:

Case History

Submitted one Killer Whale for post mortem.

Found dead and examined at Tahsis BC, tagged at base of dorsal fin.

ID: L95

Final Diagnosis

MORPHOLOGIC DIAGNOSES:

1). Dorsal fin base, tag site: Puncture wounds, moderate, bifocal, with retained tag pedicles and associated superficial dermal discoloration, necrotizing transmural vasculitis with florid intralesional fungi hyphae morphologically consistent with mucormycosis, multifocal edema fluid and cellulitis
2). Abdominal cavity: Peritonitis, moderate, multifocally extensive, fibrinous, acute with massive splenomegaly (Gross diagnosis)
3). Right costal arch and paralumbar fossa: Hematoma, subcutaneous and muscular, moderate, focal (Gross diagnosis)
4). Skin, subcutis: Microcavitations, moderate, multifocal to coalescing
5). Lung: Bronchopneumonia, moderate, multifocal with bronchiectasis with abundant liquefactive necrotic debris, florid intralesional hyphae with extension into the adjoining interstitium and focal, circumferential vascular transmural fungal hyphae morphologically consistent with Zygomycetes (mucormycosis)
6). Liver: Cholestasis, moderate, multifocal, ductular and canalicular with numerous variably sized parenchymal microcavitations
7). Presumptive hypodermis: Cytoplasmic condensation, fat cells, moderate, multifocal to coalescing, with variable interstitial edema fluid, numerous microcavitations, and globular proteinaceous deposits
8). Vascular rete: Microcavitations, stromal, moderate, multifocal with scattered acellular proteinaceous material (possible edema fluid)
9). Kidney: Mineral deposition, medullary, intratubular, mild to moderate, multifocal, random
10). Skeletal muscle: Myocellular degeneration, segmental, moderate, with endomysial proteinaceous deposits and numerous microcavitations which occasionally contain acellular refractile to finely granular proteinaceous deposits
11). Blubber: Edema, mild to moderate, multifocal, random with occasional fat cyst formation
12). Skin: Denuded, moderate, diffuse with florid superficial laminar accumulation of extracellular large blunt rod bacteria

morphologically consistent with Clostridium spp
13). Larynx: Microcavitations, adventitial, moderate, multifocal to coalescing
14). Teeth: Fracture, oblique, focal, with multifocal tooth laxity and wear (Gross diagnosis)

There are no significant lesions within the peripheral nerves, peripheral vasculature, adipose tissue, colon, small intestine, testes or brain.

Blubber margins, excised implant site (Cr, M, Ca):
1). Cranial:
i). Dorsal: Bacterial overgrowth, interstitial, mild, multifocal, random with scattered stromal dissolution
ii). Mid: Bacterial overgrowth, moderate, multifocal with stromal fragmentation
iii). Ventral: Bacterial overgrowth, mild, multifocal with diffuse contraction and wavy bands throughout the fibrous connective tissue

2). Middle:
i). Dorsal: Bacterial overgrowth, mild, multifocal, random with wavy bands throughout the stroma
ii). Mid: Bacterial overgrowth, intravascular and interstitial, marked, disseminated, with multifocal stromal dissolution and acellular proteinaceous lakes
iii). Ventral: Bacterial overgrowth, minimal, multifocal, random with scattered stromal dissolution

3). Caudal
i). Dorsal: Bacterial overgrowth, mild, multifocal, random with scattered stromal fragmentation
ii). Mid: Bacterial overgrowth, moderate, multifocal with stromal fragmentation and occasional proteinaceous lakes
iii). Ventral: Bacterial overgrowth, minimal, multifocal, random with multifocal stromal dissolution and fat cell cytoplasmic condensation

Tag sites, cauda (numeric labelling):
The distance between the centers of the cranial and caudal tag sites was 3.5 cm and the longitudinal elliptical grey discoloration of the exposed dermis was 10.0x7.5 cm. For the caudal tag, the retained petals were dissected out by perpendicular incisions to the tag site and a series of 19 tissues were harvested around and deep to the implant site.

Slide 1, surface margin, implant site: There are two samples of blubber, along the margin of 1 sample, there is a small calibre blood vessel which centrally contains faintly eosinophilic homogenous material admixed with numerous fungal hyphae that extend multifocally across the vessel wall and into the adjoining stroma. In the second tissue sample, there is multifocal hypo to acellular proteinaceous material interposed within the fibroadipose tissue and multifocally admixed with small numbers of fungal hyphae and along 1 margin of the tissue, there is a dense linear aggregate of fungal hyphae admixed with small amounts of proteinaceous debris.

Tag sites, cranial (alphabetic labelling):
For the cranial tag wound, the excised block of dorsal fin was subsampled into 5 blocks of tissues, A-E with the implant site along the caudal margin of block C. Twenty four tissue cassettes were collected with samples dissected from the immediate dorsal, cranial, ventral and caudal margins of the implant sites as well as adjoining tissues and sampled deep, medial and ventral to the tag site.

Slide CX: Throughout the stroma, there is multifocal to coalescing lakes of hypocellular proteinaceous material occasionally admixed with variable numbers of fungal hyphae; deep within the stroma, there are scattered small to intermediate calibre blood vessels which are moderately distended with variable refractile to finely vacuolated proteinaceous material admixed with numerous fungal hyphae which multifocally extend across the vessel wall and occasionally track a short distance into the adjoining fibroadipose tissue (blubber).

COMMENTS:

Post mortem change hampered microscopic review of sectioned tissues and precluded evaluation of multiple levels of the gastrointestinal tract; the most significant histopathology findings were in the lung and tag implant site. As part of the dissection, the tissue block spanning the base of the dorsal fin and encompassing the two tag implant sites was harvested and preserved en mass in formalin. The block was 19.5 cmx 20.0 cm at the base and the cranial base was 10.0 cm wide and the caudal was 5.0 cm wide. The tissue was symmetric with bilateral concavity and tapered dorsally and caudally. Radiography disclosed retained tag petals within the blubber of the cranial and caudal tag sites. There was no apparent host inflammatory response at the site on initial review of x-rays and to better define the petal location, depth of penetration and more subtle host response, magnetic resonance images (MRI) was pursued and images forwarded to two board certified veterinary radiologists for consultation (Drs. S. Dennison and T. McKlveen). These images studies confirmed two penetrating wounds, which extended 5.7 and 6.4 cm deep into the blubber with mild edema, hemorrhage, hematoma, or inflammation in the more superficial tissues. Deeper in the soft tissue, the tracts are associated with deviation of blood vessels and although the petals impinged upon ramifying blood vessels, there is no indication of vascular incision.

After review of the radiographs and MRI studies, the block of tissue was initially dissected and labelled into 5 subsections of tissues and representative samples were collected and labelled from the entrance margins of both tag implant sites, lateral and deep margins, as well as systematically through the remaining block. Although imaging studies revealed that the penetrating tracts extended 5.7 and 6.4 cm into the defects, the petals in the caudal and cranial implant sites were 1.3 cm and 1.8 cm deep, respectively. The diameter of cranial entrance site was 1.0x1.0 cm and caudal was 1.0x1.4 cm and both wounds tapered deep into the tissues. The tag shafts are 6.5 cm and the petals are positioned as two circumferentially radiating arrays at different levels along the barb shafts, which may account for the results from images studies, tissue dissection and level of petal recovery. The most salient findings associated with the penetrating wounds were superficial bacterial colonization of the defect and a myriad of nondichotomously branching, pauciseptate fungal hyphae morphologically consistent with mucormycosis. The fungal elements extended transmurally into multiple intermediate calibre thick walled blood vessels in the tissues adjoining and dorsal to the implant sites and tracked along planes of fibrovascular stroma. There was sparing of lymphatics, veins and venules. Involved blood vessels extended up to 2.0-3.0 cm from the implant sites. The more superficially involved vasculature comprised the counter current exchange vessels; with a host inflammatory response and increased physical activity, redistribution of blood and augmented vascular perfusion of the dermal and superficial subcutaneous blood vessels would have occurred, which may have enhanced deeper tissue fungal invasion and possible dissemination to other anatomic sites.

Deep within the lung parenchyma, there were intermediate to large caliber thick walled blood vessels with luminal and transmural fungal hyphae morphologically consistent with the tag site. Based on the nature of the necrosis and stage of inflammatory cell recruitment in the lung, fungal invasion of the blood vessels and bronchopneumonia most likely occurred post tagging. Localization of fungal hyphae to the lung was presumably hematogenous, although inhalation or deeper tissue invasion of hyphae from the tracheal mucosa may be a consideration. In the lumen of a small number of bronchi, there were scattered fungal hyphae with associated hemorrhage and inflammatory infiltrate which may reflect direct extension from the vasculature and adjoining interstitium. Due to the degree of putrefaction, a specific cause of death could not be assigned to L95; however, the possibility of mycotic emboli or thromboembolism to the heart, brain or other vital tissue cannot be discounted.

The morphology of the fungal hyphae was consistent mucormycosis (zygomycosis), which comprise a number of genera, including Absidia spp., Rhizopus spp., Mucor spp., Apophysomyces spp., Cunninghamella spp., and Mortierella spp. These organisms are most commonly associated with detritus and soil and infection in terrestrial animals and humans is usually opportunistic, secondary to traumatic penetrating wounds or tissue damage or associated with generalized debilitation or immunosuppression. In some cases, infections are confined to the skin; whereas, in other instances, systemic infections can occur. Mucormycosis has previously been reported in a wild juvenile southern right whale stranded in South Africa with hyphae localized to the epaxial skeletal muscle and in display Atlantic bottlenose dolphins in Florida. There are three reports of killer whale infections with zygomycosis (mucormycosis). A killer whale presented from the Vancouver Aquarium with generalized fungemia and infarction; a second killer whale infected with Sakasenaea vasiformis had lung, uterus and brain involvement and a third killer whale presented with encephalitis. In other small cetacean species, disseminated, pulmonary, cutaneous and gastrointestinal infections, often with regional lymph node involvement have been described. Gastric zygomyces have been identified in stomach contents of display animals and animals may present after bouts of intercurrent disease, prolonged antibiotic administration and other factors. Gastrointestinal involvement was not apparent in L95.

In the case of L95, it is difficult to assign a sequential pathogenesis or mechanism of fungal involvement; this animal may have been immunosuppressed or debilitated due to other factors prior to tagging and subsequent death. Before the tag was deployed, L95 was visually assessed through pacing the whale and there was no indication of suboptimal health, including depression behind the blowhole or apparent rib impressions. Follow-up review of photos obtained at the time of tagging confirmed the absence of a nuchal depression or prominent ribs. However, images captured 3 days later disclosed rib outlines. While this plane of nutrition may be considered suboptimal during the summer season, it is not uncommon to observe a similar loss of condition in the winter. A decline in nutritional status was observed in other southern resident killer whales that were present at the time L95 was tagged and has been noted in animals in previous years. At necropsy, the carcass was deemed moderate to fair nutritional status and microscopically, adipocytes were replete with fat. In addition to the nutritional status of L95, elevated contaminates and intercurrent disease such as the peritonitis and splenomegaly may have predisposed or possibly exacerbated the fungal infection in this animal. Although microscopic review of the testes was impeded due to post mortem change, the lack of spermatogenesis may represent a seasonal variation in sperm production, intercurrent disease, age of the animal, and other factors.

Molecular studies proved negative for Morbillivirus and Brucella spp and the possibility of false negative results due to tissue degradation cannot be discounted. Microbiology isolates from harvested tissues are consistent with putrefaction. Few to light Enterococcus spp and Granulicatella balaenopterae were cultured from the lung; G balaenopterae was first recovered in a minke whale that stranded on the northwest coast of Scotland. In humans, Granulicatella spp infections have been rarely associated with abdominal lesions, bacteremia and endocarditis. No bacteria were isolated from the spleen, swab or skin. The Enterococcus spp and Psychrobacter spp recovered from the colon and small intestine are likely normal flora and no Salmonella spp, Campylobacter spp, or Yersinia spp were isolated in selective media from the small intestine.

There was heavy growth of Clostridium novyi and C septicum from the skin by anaerobic culture. These microbes are presumably invaders, secondary to the penetrating tag wounds. In domestic animals, C novyi is associated with gangrene and black disease and C septicum is the etiologic agent of malignant edema (wound infections) in farm animals. Immunofluorescence for C sordellii, C septicum, and C chauvoei toxins proved negative and positive for C novyi. It is possible that secondary involvement of C novyi may have contributed in part to the pathogenesis of the tag wound lesions. The extent of tissue decomposition precluded further ancillary diagnostic efforts.

The ductular and canalicular cholestasis is suggestive of an extra-hepatic obstruction of the bile duct, possibly related to peritonitis, although increase production or failure to conjugate bile, bilirubin or bile constituents may be considerations. Endotoxin induced cytokines, toxin exposure and other factors may have contributed to this process as well. Histopathology of the spleen revealed widespread autolysis with little cellular detail or architecture. Although a cause of the massive splenomegaly could not be determined microscopically, fungemia and fibrinous peritonitis are suggestive of antigenic stimulation and reactive change. The fibrinous peritonitis was acute and likely terminal; this process is commonly recognized in stranded killer whales and may be septic or aseptic. The intratubular mineral deposition in the kidney is consistent with dehydration, although vitamin A, heavy metal, chronic inflammation, intercurrent infectious disease and other processes may also be considered.

In summary, L95 presented in an advanced state of autolysis with two penetrating tag wounds along the base of the dorsal fin, splenic enlargement, and acute peritonitis. Serial sections of the entrance wounds disclosed transmural vasculitis with invasive fungal hyphae morphologically consistent with mucormycosis. Similar hyphae are evident within blood vessels and infiltrating adjoining tissue and airways in multiple lung sections suggesting initial fungal invasion of the skin, with subsequent dissemination to the lung and possibly other tissues. The proximate cause of death may be attributed to the relatively deep tissue perforation with the tag deployment and the ultimate cause of mortality is disseminated mucormycosis. Despite multiple attempts the fungus could not be cultured or speciated by molecular means from fresh and formalin fixed tissues. It could not be conclusively determined whether the fungus may have been introduced to the tag site by a contaminated tag or if the fungus may have colonized the skin prior to tag deployment, with introduction of hyphae into deeper tissues either via tracking the tag shaft or the retained petals acting as nidi of infection and inflammation. On February 23, 2016, the tag had initially been deployed, missed the animal, was recovered from the water, immersed in disinfectant then reused with no sterilization. It is possible that this breach in protocol may have resulted in deployment of a contaminated tag barb and petals. Fibrinous peritonitis and splenomegaly have previously been diagnosed in killer whales that have not been tagged and most likely represent a septic process and in this case, it is difficult to assess the impact of tag deployment, detachment and retained petals to the abdominal inflammation.

Necropsy

A 20 year old male killer whale (Orcinus orca) with minimal reproductive activity is presented dead April 1, 2016 in poor post mortem (code 3.5) and fair to moderate body condition. There is generalized loss of the epidermis with only small tags of epithelia along the dorsolateral aspect of the torso. The exposed dermis is mottle pale tan red to yellow with multifocal dermal rake marks and occasional 6-8 cm diameter elliptical ulcerations. The ventral aspect of the thorax and cranial abdomen are dry and the stroma is prominent. The thoracic cavity is markedly gas inflated with associated distention and attenuation of the blubber. On cut section, the blubber is dull tan yellow to brown red, oozes little oil and the stroma is prominent. The right second to last mandibular tooth is obliquely fractured with the lingual aspect more prominent and the caudal 5 teeth of the upper jaw are absent. There are multiple teeth with mild staining and wear with laxity in the alveolus. The dorsal surface of the tongue is macerated. At the caudal third level and at the base of the right lateral aspect of the dorsal fin, there is a well delineated 15x10 cm elliptical pale grey discoloration of the exposed dermis which eccentrically encompasses two penetration wounds 3.5 cm apart and perpendicular to the skin surface. The more cranial defect is 1.4 cm in diameter and 1.9 cm deep and the more caudal is 1.8 cm in diameter and 1.3 cm deep; along the caudal margin of the cranial defect, there is a transverse sheared elongated lanceolate tag pedicle that conforms to the contour of the defect. On digital palpation of the penetrating wounds, the margins are mucoid to firm and extend approximately 2 cm deep. At the midlevel of the right costal arch, there is focally extensive subcutaneous hemorrhage with necrosis and shearing of the adjoining abdominal musculature. The midventral caudal abdomen is perforated with multiple loops of collapsed bowel and mesentery exteriorized and partially macerated. The sheath of the penis features numerous elongate furrows and the penile mucosa is mucoid and exfoliated (artifact). Immediately caudal to the anus, there is a large, transverse hemi-elliptical defect which extends up to 15 cm deep into the torso; the leading margins are serrated and more abrupt whereas, the more caudal limits are gradual and smooth. On incision of the abdominal cavity, the serosal surfaces of multiple loops of bowel are attached and readily reduced by digital manipulation; on release of the affected segments of intestine, there is a fine pale yellow to red granularity of the serosal surface (fibrin deposits). There is massive enlargement of the spleen (approximately 30 cm diameter) which on cut surface is glistening, slightly protuberant and homogeneous red black. There is a small amount of turbid red brown ingesta in the stomach with detached keratinising stratified epithelia and the small intestine contains a few partially digested fish bones interspersed within tan brown to yellow chyme. There are segments of bowel with submucosal gaseous distention of the mucosa with rare 0.4-0.6 cm flat elliptical black deposits.

Biological Data (please refer to the DFO marine mammal incident response form, affixed):

Total length 21'9.9"
Blubber thickness, thoracic midline
Dorsal 6.8 cm
Lateral 4.5 cm
Midventral 3.4 cm

GROSS DIAGNOSES:

1). Dorsal fin base: Puncture wounds, moderate, bifocal, with retained tag pedicles and associated superficial dermal discoloration
2). Abdominal cavity: Peritonitis, moderate, multifocally extensive, fibrinous, acute with massive splenomegaly
3). Right costal arch and paralumbar fossa: Hematoma, subcutaneous and muscular, moderate, focal
4). Teeth: Fracture, oblique, focal, with multifocal tooth laxity and wear

COMMENTS:

This animal presented in very poor post mortem condition with gaseous distention of the thoracic cavity, caudal perforation of the midventral abdominal wall, herniation of multiple loops of bowel and widespread loss of the epidermis. Two puncture wounds corresponding to tag implant sites were noted along the right lateral aspect at the base of the dorsal fin and a detached tag petal was contoured around the caudal margin of the more caudal defect. Based on the degree of putrefaction, gross examination deemed the carcass as moderate nutritional condition. However, discussion with Dr B Hanson, NOAA revealed that at the time of darting on February 23, 2016, the animal appeared in good condition. Follow up observation on February 25, 2016 indicated that the whale appeared thin with observed rib outlines and active. These observations were consistent with other southern killer whales transiting the region this season. L95 was also observed alive February 24, 2016; however, the right side of the animal could not be approached to assess the tag site. Transmission was lost with presumptive tag detachment Feb 26, 2016. Based on the nature of the peritonitis and reactive change in the spleen it is difficult to establish a precise time line in the pathogenesis of the inflammation relative to tag deployment. The dorsal fin was dissected and excised. The tag implant site block is rectangular and tapering with contours corresponding to the insertion of the dorsal fin. The excised sample is 22.5 cm long, 17.8 cm height and the base is 19.5x20 cm. The more cranial and wider margin is 10.0 cm and the caudal tapering border spans 5.0 cm. Initial tissue sections were collected from the cranial, midlevel and caudal borders of the block and 3 samples were harvested from the dorsal, mid-lateral and basal regions for histopathology evaluation. X-rays of the excised block revealed 4 haphazardly arranged pedicles in the cranial tag site and 3 petals arranged as a rosette and centred on the caudal implant site. A more detailed dissection of the implant sites is underway and additional details are to follow. The right costal and paralumbar subcutaneous hematoma was likely related to agonal or terminal impact with the substrate.

Histopathology

Refer to Morphologic Diagnoses

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Hughes,Giselle on 04/06/16 @ 11:34 AM

Specimen	ID	Isolate	Result	Level
Lung		Enterococcus sp.	Positive	Few
Lung		Bacteria	Positive	1+
Bacteria identified as Granulicatella balaenopterae by DNA sequencing.				
Spleen			No Bacteria Isolated	
Swab	Tag implant site		No Bacteria Isolated	
Skin			No Bacteria Isolated	
Colon		Enterococcus sp.	Positive	2+
Colon		Psychrobacter sp.	Positive	4+
Small Intestine		Enterococcus sp.	Positive	4+

Anaerobic Culture - Prod Resulted by: Erin Zabek Verified by: Hughes,Giselle on 04/08/16 @ 11:14 AM

Specimen	ID	Isolate	Result	Level
Skin		Clostridium novyi	Positive	4+
Skin		Clostridium septicum	Positive	4+

Culture - Campylobacter Resulted by: Erin Zabek Verified by: Hughes,Giselle on 04/07/16 @ 9:32 AM

Specimen	ID	Isolate	Result	Level
Small Intestine			No Campylobacter sp. isolated	

Culture - Yersinia Resulted by: Erin Zabek Verified by: Hughes,Giselle on 04/07/16 @ 9:32 AM

Specimen	ID	Isolate	Result	Level
Small Intestine			No Yersinia sp. Isolated	

Culture - Salmonella Resulted by: Erin Zabek Verified by: Hughes,Giselle on 04/07/16 @ 9:32 AM

Specimen	ID	Isolate	Result	Level
Colon			No Salmonella sp. Isolated	
Small Intestine			No Salmonella sp. Isolated	

FA - C. chauvoei Resulted by: Hughes,Giselle Verified by: Erin Zabek on 04/06/16 @ 11:11 AM

Specimen	ID	Test	Result
Skin		FA - C. chauvoei	Negative

FA - C. novyi Resulted by: Hughes,Giselle Verified by: Erin Zabek on 04/06/16 @ 11:12 AM

Specimen	ID	Test	Result
Skin		FA - C. novyi	Positive

FA - C. septicum Resulted by: Hughes,Giselle Verified by: Erin Zabek on 04/06/16 @ 11:12 AM

Specimen	ID	Test	Result
Skin		FA - C. septicum	Negative

FA - Clostridium sordellii Resulted by: Hughes,Giselle Verified by: Erin Zabek on 04/06/16 @ 11:12 AM

Specimen	ID	Test	Result
Skin		FA - Clostridium sordellii	Negative

GPOS Resulted by: Erin Zabek Verified by: Hughes,Giselle on 04/07/16 @ 9:33 AM

	Organism
Antibiotics	Enterococcus sp.
Enrofloxacin	s
Erythromycin	s
Gentamicin	s
Lincomycin	r
Penicillin G	s
Sulphamethoxazole/Trimethoprim	r
Tetracycline	s
Florfenicol	s

Molecular Diagnostics

DNA Extraction Resulted by: Verified by: Tomy Joseph on 04/05/16 @ 4:14 PM

Specimen	ID	Test	Result
Tissue	sk.musc,tong,diaph,lv	DNA Extraction	

Further testing by NIH.

Stephen Raverty, DVM
Stephen.Raverty@gov.bc.ca

These results relate only to the animals or items tested.
This report shall not be reproduced except in full, without the written approval of the laboratory.
The above material is intended only for the use of the individual to whom it is addressed, as it may contain confidential or personal information that is subject to provisions of the Freedom of Information and Protection of Privacy Act. This material must not be distributed, copied or disclosed to other unauthorized persons. If you have received the transmission in error, please contact the sender immediately by telephone.

END OF REPORT