Necropsy Report
Killer Whale (Orcinus-orca) Kanduke
Age 25 yrs — SeaWorld of Florida

Name: Kanduke (male) (aka Kandu IV, Kandu 4, T9, T009)

Species: Killer Whale (Orcinus orca)

Source: wild capture, 08-16-1975, Pedder Bay, BC, Canada, age: est. 5 yrs

Deceased: 09-20-1990, SeaWorld of Florida, age: est. 25 yrs

Reported cause of death (per NMFS MMIR data): Viral Leptomenigitis

Necropsy info:
Histology- Mike Walsh, DVM, SeaWorld (1990):
Histologic and bacterial examination of tissue have not pointed to an exact cause of death, though the opinion of the independent histopathologists who conducted the examinations is that a virus is the most likely cause. Additional independent studies including virus isolation and further histologic examination will be undertaken in order to attempt to pinpoint a virus as a cause of death. If additional pertinent information is obtained, it will be added to the necropsy report.

A gastric foreign body was found in the first compartment of the animal's stomach. While the presence of the object (a 55 x 20 x 13 cm collapsed fishing buoy) was visually striking, it was not related to the cause of this animal's death. This object did not appear to impede normal food intake or digestion. Since there was no chance of access to this object while at SeaWorld, it had to have been present prior to the time of the animal's arrival at SeaWorld of Florida.

Initial results from first pathologist on 10-12-90 pointed toward a possible viral infection as evidenced by:
1) Meningoencephalitis
2) Lymphoid hyperplasia
3) Enteritis
A second set of tissues were submitted to a second histopathologist. Initial phone results indicated similar findings and additional slides containing meninges were sent by the 1st pathologist for review. The second histopathologist's major findings included:
1) Leptomeningitis
2) Chronic cerebral and spinal chord perivasculitis
3) Lymphoid inflammation of intestinal tract

**Attempts at virus isolation included:**
- Sam H. Ridgway DVM, NOSC (1991)
- Charles D. Buck, Animal Virus Collection Manager, American Type Culture Collection (1991)

**1993 Update:**
Although not reflected or updated in NMFS MMIR, a 1993 study was published—“Isolation of St. Louis encephalitis virus from a killer whale” which indicates the death of Kanduke was caused by the St. Louis encephalitis virus with a mosquito vector. That report is attached at the end of this document.

The 1993 published paper was co-authored by:
- Charles Buck & Grace P. Paulino - Virology Department, American Type Culture Collection
- Daniel J. Medina & G.D. Hsiung - Department of Laboratory Medicine, Yale University School of Medicine & Virology Laboratory, VA Medical Center
- Terry W. Campbell & Michael T. Walsh - SeaWorld of Florida, Orlando, FL

**More on the mosquito vector and virus:**

Whale and Dolphin Conservation Society:
*Documents Prove Mosquito-borne Virus Responsible for Captive Orca Death*

Voice of the Orcas:
*Mosquitoes have killed 2 SeaWorld Orcas: Has Anyone Noticed?*
[https://sites.google.com/site/voiceoftheorcas/home/the-current-story/mosquitoeshavekilled2seaworldorcashastheanyonenoticed](https://sites.google.com/site/voiceoftheorcas/home/the-current-story/mosquitoeshavekilled2seaworldorcashastheanyonenoticed)

Whale and Dolphin Conservation Society:
*WDCS And Partners Reveal Unseen Threat To Orcas In Captivity*

Voice of the Orcas:
*John Jett & Jeffrey Ventre reveal "Death by Mosquito" at Marine Mammal Conference with WDCS*
[https://sites.google.com/site/voiceoftheorcas/home/the-current-story/johnjettjeffreyventrerevealdeathbymosquitoat marinemammalconferencewith wdc](https://sites.google.com/site/voiceoftheorcas/home/the-current-story/johnjettjeffreyventrerevealdeathbymosquitoat marinemammalconferencewith wdc)
Poster presented by Dr. John Jett at 2012 Marine Mammal Health Conference-Sarasota, FL:  
*Evidence of Lethal Mosquito Transmitted Viral Disease in Captive Orcinus orca*  
http://www.wdcs-na.org/submissions_bin/fmmhc.pdf

**Notes:** Prior to reforms of the Marine Mammal Protection Act (MMPA) in 1994, holders of marine mammals for public display were required to submit necropsy reports (animal autopsy reports) for deceased animals, making the documents available to the public and scientific community. Presently, marine mammal parks in the U.S. are only required to provide a “cause of death” to the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) which maintains Marine Mammal Inventory Reports (MMIR). Details of marine mammal deaths are now a closely guarded secret at U.S. entertainment facilities.

The Orca Project acquired the following documents from the National Marine Fisheries Service (U.S.A.) via the Freedom of Information Act for deaths that occurred prior to implementation of the 1994 MMPA changes.

For more information visit [www.theorcaproject.com](http://www.theorcaproject.com)

Dear Dr. Foster:

Please find enclosed the pathology report from our 25+ year old killer whale 00-8701, who died on September 20, 1990. As you are aware, this animal had been maintained at Sea World of Florida for three years since his arrival from Marineland of Canada. The animal had a normal consistent food intake during his stay at Sea World, with no apparent gastrointestinal problems. Age was estimated at 25+ years by counting growth-layer-groups in an acid-etched, longitudinally bisected tooth.

After a normal breeding period from September 15-17, 1990, the animal showed a decrease in appetite followed by a rapid deterioration of its clinical appearance (see history on pathology report) and death on September 20, 1990.

Histologic and bacterial examination of tissue have not pointed to an exact cause of death, though the opinion of the independent histopathologists who conducted the examinations is that a virus is the most likely cause. Additional independent studies including virus isolation and further histologic examination will be undertaken in order to attempt to pinpoint a virus as a cause of death. If additional pertinent information is obtained, it will be added to the necropsy report.

A gastric foreign body was found in the first compartment of the animal's stomach. While the presence of the object (a 55 x 20 x 13 cm collapsed fishing bouy) was visually striking, it was not related to the cause of this animal's death. This object did not appear to impede normal food intake or digestion. Since there was no chance of access to this object while at Sea World, it had to have been present prior to the time of the animal's arrival at Sea World of Florida.

Sincerely,

Michael T. Walsh, D.V.M.
### MARINE MAMMAL COLLECTION/INVENTORY REPORT

**NAME OF ANIMAL HOLDER:** Sea World, Inc.  
**DATE OF REPORT:** 11/19/90

**SPECIES SCIENTIFIC NAME:** Orcinus orca  
**COMMON NAME:** Killer whale

<table>
<thead>
<tr>
<th>ANIMAL NAME/IDENTIFICATION</th>
<th>SEX</th>
<th>EST BIRTH YEAR</th>
<th>AUTHOR DOCUMENT</th>
<th>DATE TAKEN OR ACQUIRED</th>
<th>TAKE TYPE</th>
<th>LOCATION OF TAKE PLACE NAME AND LATITUDE-LONGITUDE</th>
<th>COLLECTOR OR SOURCE</th>
<th>CURR STAT</th>
<th>DEATH OR DISPOSITION DATE EXPLANATION</th>
<th>NECRP FILED NMFS</th>
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<tr>
<td>SWF-00-8701 M</td>
<td>M</td>
<td></td>
<td>#575</td>
<td>1-09-87</td>
<td>LM</td>
<td>From Marineland, Canada, Niagara Falls, Canada</td>
<td>N/A</td>
<td>D-C</td>
<td>9-20-90 Chronic nonsuppurative leptomeningitis, cerebral perivasculitis, intestinal lymphoplasmacytic inflammation, final diagnosis pending attempts at virus isolation and additional review of brain and intestinal tract tissues</td>
<td>Yes</td>
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</tbody>
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SEA WORLD
GROSS NECROPSY REPORT

FACILITY: Sea World of Florida  PROSECTOR: Walsh, Campbell, Buesse, Odell

GENUS/SPECIES: Orcinus orca

ID NUMBER: 00-8701  AGE: 25+  SEX: Male

DATE OF DEATH: 9-20-90  DATE OF NECROPSY: 9-20-90

EXTERNAL MORPHOMETRICS: (metric only)

WEIGHT: 5,568 kg

TOTAL LENGTH: 675 cm  GIRTH AT AXILLA: 

GIRTH AT ANUS: See data sheet  FLUKE WIDTH: 

GIRTH AT UMBILICUS:  DORSAL FIN HEIGHT: 

HISTORY:
This mature male killer whale was obtained from Canada for Sea World's breeding program in 1987. Upon arrival, the animal was noted have numerous worn teeth which did not seem to inhibit eating or normal activity. His overall behavior during this period was similar to breeding males of other species with periods of active involvement with the females during heat cycles followed by separation by the females during non-receptive periods. His health was stable with an occasional slight rise in his serum creatinine. Because of his potential age, dietary adjustments were made by the veterinary staff to avoid age related kidney disease as seen in most older mammals. Following a heavy breeding episode from 9-15-90 - 9-17-90, there was a slight decrease in his appetite and he avoided his caretakers. Attempts at obtaining a pool side fluke blood sample were unsuccessful, so the animal was placed in the medical pool. At this time it was noted that his attitude had rapidly deteriorated. Antibiotics were administered after the blood sample and the water returned to normal levels without incident. Before his planned second treatment it was noted that he was incoherent and not bothering to right himself in the water column. Assistance was provided by use of a stretcher to avoid water inhalation but the animal was nonresponsive and died shortly thereafter.
**GENERAL EXTERNAL APPEARANCE:** (oral cavity, external nares, skin, eyes)
The animal's overall weight appeared grossly normal. The majority of the teeth in all four archades were chronically worn but showed no evidence of infection.

**SUBDERMAL CONDITION:** (blubber, muscles, lymph nodes)
Blubber layers were adequate. There were no gross abnormalities in the muscle groups. External lymph nodes were nonremarkable. Refer to morphometric data sheet.

**CRANIAL EXAM:** (ears, melon, pterygoid sinus)
Ears - no gross abnormalities
Melon - 

Pterygoid sinuses were clean

**CENTRAL NERVOUS SYSTEM:** (brain, pituitary, spinal cord)
Brain - Sections were taken for histology and frozen. The brain was fixed in formalin for possible CAT scan and MRI. No gross lesions were noted.

**THORACIC CAVITY:** (pleura)
No gross lesions were found and the pleura was clean. There was no gross accumulation of fluid in pleural cavity.

**UPPER RESPIRATORY SYSTEM:** (nasal sacs, nares, larynx)
An ulcerative lesion was found in the caudal exterior portion of the exterior blow hole. This appeared to be limited to a 1.5 x 3cm area where the blow flap contacts to the caudal wall. No other portion of the nasal passage was affected.

**LOWER RESPIRATORY SYSTEM:** (trachea, bronchi, lungs, lymph nodes)
Tracheal epithelium was brown in color.

**CARDIOVASCULAR SYSTEM:** (heart, aorta, major vessels)
No gross abnormalities were noted, valves were clean, and no myocardial discoloration was noted.
ABDOMINAL CAVITY: (lymph nodes)
Abdominal lymph nodes appeared slightly enlarged. No abnormal fluid was present.

DIGESTIVE SYSTEM: (esophagus, stomach, intestine, cecum, rectum, lymph nodes)
There was a circular band of mucosal ulceration in the caudal portion of the esophagus. Cytology was negative for Candida. The stomach contained a 55 x 20 x 13cm deflated, brittle, fishing buoy which was discolored dark brown. Raised lettering was present on the buoy. The buoy apparently originated from a Norway company. A dozen small stones and a small piece of wood 2 x 8cm were found in the stomach. No ulcerations were present in the stomach chambers. Numerous 1-2mm white circular areas were diffusely scattered through the duodenum resembling peyers patches.

LIVER: (biliary system)
The liver tissue was normal in color though the serosa was more opaque and thickened as is seen in older animals. The texture of the liver parenchyma appeared fibrotic.

PANCREAS:
No gross lesions.

Spleen:
No gross lesions, serosa was thickened.

REPRODUCTIVE SYSTEM: (testicles, ovaries)
A normal mature male reproductive tract was found. A penile scar 10 cm long was present on the left lateral surface, with no obvious problems noted.

URINARY SYSTEM: (kidneys, ureter, bladder, urethra)
No gross lesions noted in any segment. Kidneys were normal in color and the urinary bladder contained 20cc of urine. Urine color was straw colored.

ADRENAL GLANDS:
No gross lesions.

SKELETAL SYSTEM:
No gross lesions.

PARASITE SUMMARY
None observed.

SPECIAL TESTS
Tissues were taken for virus isolation, metal and pesticide analysis.
GROSS SUMMARY

1) Ventral pneumonitis - acute
2) Esophagitis - ulcerative - chronic
3) Gastric foreign body - chronic

TENTATIVE DIAGNOSIS:

Open until bacteriology and histology. The rapid clinical decline of this individual would suggest an infectious or toxic influence.

CONCLUSIONS: (after histology & clinical pathology review)

See attached sheet.

DATE: 4-26-90  SIGNED: Michael J. Hall, D.V.M.
Conclusions  

Bacterial: There was no evidence of septicemia from tissue bacterial culture. Normal bacteria were found in the upper respiratory and colon area.

Histology: Initial results from first pathologist on 10-12-90, pointed toward a possible viral infection as evidenced by
1) Meningoencephalitis
2) Lymphoid hyperplasia
3) Enteritis

A second set of tissues were submitted to a second histopathologist. Initial phone results indicated similar findings and additional slides containing meninges were sent by the 1st pathologist for review.

The second histopathologist's major findings included
1) Leptomeningitis
2) Chronic cerebral and spinal chord perivasculitis
3) Lymphoid inflammation of intestinal tract

Additional tissues were requested on 11-13-90 which will be sent for processing.

CAT and MRI exam of brain
- No lesions were noted.

Comments: The acute clinical decline of this animal suggested the possibility of an infectious or toxic etiology. The relative absence of any lethal lesions noted at necropsy also suggested that the diagnosis would depend on the histopathology evaluation. Findings by independent histopathologists resulted in similar findings with emphasis placed on the intestinal tract, brain and lymphoid tissue.

Unfortunately if the lesions noted are viral in origin, discovery of the exact virus may be difficult. Frozen tissues will be submitted for viral isolation. Additional examination of the brain tissue will be initiated by a third party in an attempt to locate other potential lesion sites.

The ventral pneumonitis and esophagitis while evident grossly were not serious enough to be related to the cause of death. The foreign body recovered from the first compartment of the stomach, while visually surprising, did not cause the death of the animal. This is further evidenced by the fact that it was present for at least three years without any clinical signs of gastrointestinal abnormality.

Michael C. Hall, D.V.M.
Zoological Pathology Consult  
Kent G. Osborn, DVM  
Histopathology Report

ZPC #: 90SWMX.10A  
Submitted by: Sea World, Florida  
Date Received: 10-04-90  
Date Reported: 10-12-90

ID  
Species: Orcinus orca  
Common: killer whale  
Sex: male  
Breed:  
House: 4AB701  
Age: adult

Diagnoses

1. brain, cerebrum, cerebellum, spinal cord, inflammation, perivascular infiltrates, lymphocytic infiltrate plus minimal neutrophil infiltrates, cause undetermined, possible virus, see comment.

2. meninges, inflammation, lymphoid infiltrate plus neutrophilic infiltrate

3. peripheral plus mesenteric lymph nodes, interfollicular lymphoid hyperplasia, cause undetermined, possible virus, see comment

4. spleen, lymphoid hyperplasia

5. trachea, submucosa, inflammation, mild plasma cell infiltrate plus rare neutrophils

6. intestine, possible duodenum, mucosa, fibrosis plus mild, chronic inflammation

7. intestine, possible duodenum, serosa, mild acute inflammation

8. esophagus, ulcer plus chronic inflammation, lymphocytic infiltrate plus minimal/mild neutrophil infiltrate, cause undetermined, possible gastric reflux, see comment

9. skin, blowhole?, ulcer plus moderately severe chronic active inflammation, lymphoplasmacytic infiltrate plus neutrophils, etiology undetermined

10. heart, inflammation, lymphocytic plus neutrophilic infiltrate, see comment
Microscopic Summary (slide number in parentheses)

The following tissues were examined microscopically and found to be essentially normal: brachial plexus (11,14), adrenal (13), glandular stomach (14), and skeletal muscle (9).

Lesions and/or notable microscopic findings are present in the following tissues:

- brain (cerebrum (1), cerebellum with meninges (2)) and spinal cord (2): Perivascular cuffs consisting primarily of lymphocytes with minimal or no accompanying neutrophils are present in the cerebrum section, in the cerebellar white matter and in the spinal cord gray matter. There is marked involvement of the cerebellar meninges as well. Within these lymphoid aggregates, the cells are medium-sized lymphocytes and there are rare mitotic figures.

Dx: brain, cerebrum, cerebellum, spinal cord, inflammation, perivascular infiltrates, lymphocytic infiltrate plus minimal neutrophil infiltrates, cause undetermined, possible virus, see comment.

- meninges, inflammation, lymphoid infiltrate plus neutrophilic infiltrate

- prostate (3): The prostate itself has no recognized lesions, but there is a focal aggregate of lymphocytes in the periprostatic adipose/connective tissue.

- testicle (3): There is mild/moderate spermatogenesis, otherwise no lesions are recognized (NLR).

- peripheral lymph node (4): Two lymph node sections present here have widely expanded cortex, due to a reactive interfollicular lymphoid population. Follicles are relatively inconspicuous, being compressed by the large interfollicular population to an area just beneath the cortex. The follicles are not themselves obviously reactive. The interfollicular lymphoid population consists primarily of moderate to moderately large lymphocytes, admixed with smaller numbers of small lymphocytes and of lymphoblasts. Mitotic figures are common in these areas, one or more per oil immersion field. Medullary cords have moderate lymphoplasmacytic populations.

Dx: peripheral lymph node, interfollicular lymphoid hyperplasia, cause undetermined, possible virus, see comment.
lun (5,8): There are occasional loose aggregates of small lymphocytes within the parenchymal interstitial tissue, sometimes near bronchioles. Also present are scattered pigment-laden macrophages. The submucosa of some bronchi contains mild plasma cell populations. Moderate congestion is present in the section in slide B.

duodenum (6): This section of intestine, which came from a container labeled duodenum, has a marked lamina propria population of plasma cells, as well as a lymphoid nodule in the deep mucosal lamina propria.

mesenteric lymph node (6): This node has the same appearance as the peripheral lymph nodes.

trachea (7): In this section, the superficial tracheal mucosa is missing, possibly due to artifactual loss. The submucosa contains a mild loose plasmacytic infiltrate and scattered neutrophils.

Dx: trachea, submucosa, inflammation, mild plasma cell infiltrate plus rare neutrophils

intestine, not otherwise specified (NOS) (7): This section of tissue has a smooth muscle wall of several layers and a mucosa that contains crypts lined by columnar epithelium. There are some submucosal glands with cuboidal epithelium, suggestive of Brunner's glands, evidence that this may be a section of duodenum. The mucosa is moderately heavily infiltrated by fibrous tissue which distorts the expected orientation and spacing of the crypts. In addition, there is a diffuse, mild mixed inflammatory mucosal infiltrate consisting of plasma cells, lymphocytes and neutrophils. The serosa of this section has mild inflammation, with edema and a neutrophilic inflammatory infiltrate.

Dx: intestine, possible duodenum, mucosa, fibrosis plus mild, chronic inflammation, see comment

Dx: intestine, possible duodenum, serosa, mild acute inflammation

skin, possibly blow hole (7): This skin section is ulcerated on one side and contains chronic active inflammation, consisting of lymphoplasmacytic infiltrates admixed with neutrophils in the superficial dermis, plus large aggregates of neutrophils at the surface. No etiologic agents are recognized.
Dx: skin, blowhole?, ulcer plus moderately severe chronic active inflammation, lymphoplasmacytic infiltrate plus neutrophils, etiology undetermined

Skin (10): This skin section has mild/minimal lymphoplasmacytic infiltrates in the superficial dermis.

Spleen (12): There is moderate lymphoid hyperplasia, without reactive follicles, suggesting expansion of periarteriolar lymphoid sheaths. The cell populations are morphologically similar to those described in the lymph nodes, and have a similar amount of mitotic figures.

Dx: spleen, lymphoid hyperplasia

Kidney (12): The kidney cortex has widespread moderate tubular dilatation, suggestive of a "shock kidney". Glomerular morphology varies, with often swollen, at times hypercellular glomerular tufts, and variable hypertrophy of Bowman's capsule parietal epithelium. The Bowman's capsule basement membranes are variably thickened (minimal to moderate).

Liver (13): Mild centrilobular degeneration is present in this section, otherwise NLR.

Lymph node NOS (13): Morphology is similar to that described for peripheral lymph nodes.

Esophagus (14): No epithelium is present in this section of esophagus. The luminal surface is lined by a dense cellular infiltrate which overlies densely collagenous, almost hyalinized submucosa. The cellular infiltrate consists of a pleomorphic reactive lymphoid population, in which there are scattered mitotic figures. Rare small aggregates of neutrophils are also present, generally near the luminal surface.

Dx: esophagus, ulcer plus chronic inflammation, lymphocytic infiltrate plus minimal/mild neutrophil infiltrate, cause undetermined, possible gastric reflux, see comment

Heart (9): In this heart section there is mild/moderate nuclear size variation and mild/minimal intracellular myocardial fiber pigment, probably lipofuscin. Of note are scattered aggregates of inflammatory cells that generally are placed only around the margin of the section, giving some suggestion that they are artifactual "floaters" from other tissue sections. These aggregates consist of lymphocytes and smaller numbers of
neutrophils, admixed with erythrocytes.

Dx: heart, inflammation, lymphocytic plus neutrophilic infiltrate, see comment

Comment

The disease process recognized in these sections that is most likely related to the animal's death is a widespread nonsuppurative meningoencephalitis. The predominantly lymphoid nature of the inflammatory infiltrates in the central nervous system points away from a bacterial cause, giving evidence, instead for a cell-mediated reaction. Further support for a cell-mediated immune response, which could occur in a potential virus infection, is the nature of the lymphoid hyperplasia that is described in the lymph nodes and spleen. This reaction appears to be occurring in areas that are the normal homing sites of T lymphocytes. I have seen similar reactions in other animals with systemic viral infection. As Dr. Walsh and I discussed over the telephone, definitive etiological diagnosis is difficult. I agree with Dr. Walsh's suggestion that serology testing for encephalomyocarditis virus as an important rule out. Beyond that, culture for a mystery virus can be extremely elusive. I would recommend maintaining the frozen samples of tissue (brain and lymph node in particular) at -70 degrees F until such time as culture attempts might be carried out.

From the verbal history I received from Dr. Walsh, the esophagus section with ulceration probably corresponds to the distal esophageal ulcer recognized at necropsy. The grossly recognized white tissel in the duodenum probably were the tissue of lamina proprial fibrosis described in the section present here. These may be the sites of previous inflammation and possible ulcers that have since healed. The heart section inflammation, as mentioned above, is only present along the edges, making it difficult to say with assurance that these inflammatory aggregates were actually associated with the heart. I will get recuts of this slide, and have asked Dr. Walsh to send an additional heart section.

NOTE: ADDITIONAL TISSUES SENT TO Dr. Osborn on 10-23-80 TO TRY TO VERIFY AND CLARIFY HEART FINDINGS.

Dr. Osborn reported no remarkable findings in additional heart muscle samples.
November 13, 1990

Dr. Michael T. Walsh
Sea World
7007 Sea World Drive
Orlando, FL

Dear Dr. Walsh:

I recently reviewed the histopathology of a case from the University of Miami Department of Comparative Pathology (CP-90-4540-10 glass slides) and a case identified from the San Diego Zoo (RP-4323-1 glass slide). These slides represented tissues from a reported male killer whale.

Case RP-4323 contains two sections of central nervous system tissue while CP-90-4540 contains tissues from the central and peripheral nervous systems, gastrointestinal tract including liver, primary lymphoid tissues, skeletal muscle, adrenal gland and various epithelial tissues some resembling possible superficial tissues of the nasal sac region.

As one of many consultants on this case I will review only the significant microscopic lesions.

The leptomeninges of the cerebellum of RP-4323 are characterized by moderate multifocal infiltrates of primarily lymphocytes, plasma cells, and sparse numbers of histiocytes and neutrophils. There is also mild multifocal hemorrhage. Meningeal tissue is not present with the other CNS tissue submitted.

The cerebrum and spinal cord gray matter have mild to moderate perivascular cuffing of primarily lymphocytes and plasma cells. Occasional spinal cord neurons contain lipofuscin and there is mild multifocal hemorrhage.

The single section of small intestine examined is characterized by both extensive superficial and deep lamina proprial populations of lymphoid cells including lymphoblasts, well-differentiated lymphocytes and plasma cells.
Multiple sections of lymph node and spleen are examined. Some lymph nodes are remarkably abnormal characterized by lymphoid depletion of both germinal follicles and parafollicular zones. Medullary sinuses in some nodes contain numerous histiocytes and eosinophils. One node has moderate multifocal hemosiderosis with parafollicular hyperplasia. The spleen has moderate reactive changes and focal hemosiderosis.

The liver has mild centrilobular vacuolar degeneration of hepatocytes and mild extramedullary hematopoiesis.

The first and second stomach compartments are characterized by mild to moderate multifocal superficial submucosal lymphoplasmacytic infiltrates. There is also focal mucosal erosion, vacuolar degeneration and mild lymphocytic exocytosis in one section of first stomach or distal esophagus. The third stomach compartment has similar mild chronic inflammation.

Sections of nonintegumentary epithelial tissues have moderate diffuse vacuolar epithelial degeneration with moderate associated submucosal infiltrates of primarily lymphocytes, histiocytes and plasma cells. Methenamine silver stains demonstrate numerous small septate, occasionally branching, fungal hyphae in only the superficial epithelial layers and not generally associated with inflammatory cell infiltrates.

The heart has mild multifocal interstitial fibrosis and the testicles are characterized by seminiferous tubular atrophy. Only occasional tubular lumens contain mature spermatozoa.

The section of lung examined has mild multifocal interstitial infiltrates of primarily lymphoid cells. There is also focal pulmonary hemorrhage.

The adrenal gland has moderate diffuse lipid depletion primarily of the zona glomerulosa and fasciculata.
From the tissues examined the cause of this whale's death is, at best, speculative. Additional CNS tissue with associated meninges should be examined to try to ascertain the extent and possible etiology of the chronic non-suppurative leptomeningitis and chronic cerebral and spinal cord perivasculitis. While etiologic agents are not present in these tissues, the histologic pattern is suggestive of a viral or some protozoan infections.

Additional sections of enteric tissue should also be examined to try to determine if the changes present reflect marked gut-associated focal lymphoid hyperplasia or a diffuse lymphoplasmacytic inflammatory process.

If the CNS and/or enteric lesions were extensive, functional compromise of the associated organs may have been contributory factors in this animal's death.

If you have any further questions or comments please do not hesitate to contact me.

Sincerely yours,

Gregory L. Rossant, V.M.D.
Staff Veterinarian and Pathologist
March 15, 1991

Dr. Nancy Foster
Director
National Marine Fisheries Service
Protected Species and Habitat Conservation
1335 East West Highway
Room 8268
Silver Spring, MD 20910

Re: Necropsy report/SWF-00-8701

Dear Dr. Foster:

Our letter dated November 19, 1991, which transmitted the gross necropsy and pathology reports for the Killer whale identified as SWF-00-8701, stated that additional histologic and viral studies would be conducted in an attempt to pinpoint a specific virus as the cause of death of this animal.

These studies (attached), have been completed. Visual examination of the formalin fixed brain and re-examination of other brain sites histologically were conducted by three independent investigators. A fourth independent specialist received samples of all tissues for in-depth viral screening. A causative virus was not detected by the histopathologist or isolated by the viral specialist.

Therefore, the diagnosis made in the necropsy report conclusion dated 11/13/90 remains unchanged.

Sincerely,

Mike Walsh, D.V.M.
Zoological Pathology Consult  
Kent B. Osborn, DVM  
Histopathology Report

ZPC #: 905WMX.10A  
Submitted by: Sea World, Florida  
Date Reported: 3-12-91

ID  
Species: Orcinus Orca  
Common: killer whale  
Breed:  
House: 4A8701

Diagnoses

brain, cerebrum, cerebellum, inflammation, perivascular infiltrates, lymphocytic infiltrate plus minimal neutrophil infiltrates, cause undetermined, possible virus, see comment.

Comment

Additional brain sections submitted to me by Dr. Sam Ridgway contain lesions similar to those already described in the initial report on October 12, 1990. Additional heart sections submitted to me by Dr. Mike Walsh have no recognized lesions. The initially recognized peripheral inflammatory infiltrate probably were "floaters", as suspected and suggested when those sections were initially described. With no significant differences in the brain sections, the original report stands, in which this animal's primary problem is a nonsuppurative meningoencephalitis, very likely of viral etiology.

Kent B. Osborn, DVM
March 8, 1991

Dr. Michael T. Walsh
Sea World
7007 Sea World Drive
Orlando, Florida

Dear Dr. Walsh:

I recently reviewed the histopathology of additional central nervous system tissue from a killer whale (SW-00-8701). My original pathologic impression remains essentially unchanged.

The cerebellar leptomeninges are characterized by mild to moderate multifocal infiltrates of primarily lymphocytes and occasional plasma cells and histiocytes. There is also mild multifocal meningeal congestion and hemorrhage.

The brainstem, cerebrum, and cerebellum are characterized by mild to moderate perivascular infiltrates of both large and small lymphocytes and occasional plasma cells and histiocytes. Scattered neurons contain lipofuscin. In addition, there are infrequent pyknotic neurons. This is often associated with anoxia.

The diagnosis in this case remains unchanged. The etiology of the chronic non-suppurative leptomeningitis and cerebral, cerebellar, and brain stem mononuclear perivasculitis is unknown. Infectious agents were not identified in the tissue sections examined. The histologic pattern of these lesions is suggestive of a viral infection, however distinctive viral inclusion bodies were not observed.

If you have any additional questions please do not hesitate to contact me.

Sincerely yours,

Gregory D. Bossart, V.M.D.
Pathologist
Dr. Mike Walsh  
Veterinarian  
Sea World of Florida  
7007 Sea World Drive  
Orlando, Fl 32809  
Fax: 407 363 2316

Dear Dr. Walsh:

Thank you very much for giving me the opportunity to examine the brain of your Male Orca (675 cm in length and 5568 kg) died of 20 September, 1990. I have sectioned the brain and have, as yet, found no certain abnormalities. The dura over the convexity hemispheres appeared relatively opaque and thickened compared to other normal killer whales brains that I have examined. This may have simply been the result of the whales advanced age or to infection present at death or at earlier times. There was no gross evidence of inflammation or swelling on the surface of the brain tissue. I trust that the histological specimens submitted to pathologists will clarify the issue of infection.

Sincerely,

Sam H. Ridgway DVM, PhD  
NOSC  
Code 5107  
San Diego, CA 92152  
Fax: 619 553 1355  
Tel: 619 553 1374

February 21, 1991
Dear Dr. Campbell:

We have completed our initial screening of the frozen necropsy samples you provided and have isolated no virus. This screening consists of two passages in SP-1 (dolphin) cells, PrMK (monkey) cells, MiLu (mink) cells and CE (9-10 day old chick embryos). We chose not to filter these samples prior to first passage and several (as shown below) became contaminated (as evidenced by growth of presumed bacteria on blood agar plates). Supernatants from the first passage were filtered through a 0.45 μm filter prior to second passage.

Sea World Necropsy Samples for Virus Isolation

<table>
<thead>
<tr>
<th>ATCC Sample</th>
<th>Sea World Sample</th>
<th>Tc</th>
<th>P gg</th>
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<tbody>
<tr>
<td>66</td>
<td>4A8701 heart</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>67</td>
<td>4A8701 spleen</td>
<td>BA+</td>
<td>Neg</td>
</tr>
<tr>
<td>68</td>
<td>4A8701 liver</td>
<td>BA+</td>
<td>Neg</td>
</tr>
<tr>
<td>69</td>
<td>4A8701 kidney</td>
<td>BA+</td>
<td>?BA</td>
</tr>
<tr>
<td>71</td>
<td>4A8701 duodenum</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>72</td>
<td>4A8701 brain</td>
<td>SP-1?</td>
<td>Neg</td>
</tr>
<tr>
<td>73</td>
<td>4A8701 mesenteric lymph</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>74</td>
<td>4A8701 lymph node</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>75</td>
<td>4A8701 pancreas</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>76</td>
<td>4A8701 lung</td>
<td>Neg</td>
<td>Neg*</td>
</tr>
</tbody>
</table>

* This sample has only been passaged once on CE.
BA+ Growth on blood agar.
? Cells abnormal (SP-1 on 1st passage)

Only two of the samples gave any indication that a viral agent might be present. The first passage brain for 4A8701 caused questionable cytopathic effect on the SP-1 cells but we have found neural tissues often produce toxic effect on cell culture and the second passage was normal. The second passage kidney produced questionable hemagglutination on passage 2 in CE but a third passage was normal.
We will, as part of my research, continue to test the above mentioned samples and will let you know if any virus are later isolated. Thank you for the opportunity to work with you on these samples.

Sincerely,

Charles D. Buck
Animal Virus Collection Manager
Isolation of St. Louis encephalitis virus from a killer whale

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Abstract

We report the isolation of St. Louis Encephalitis (SLE) virus from a mature male killer whale (Orcinus orca). This represents the first isolation of SLE virus from a marine mammal. The animal presented with reduced appetite, rapidly became lethargic and subsequently died. Virus-induced CPE was observed in a dolphin cell line, SP-1K (ATCC CCL 78), inoculated with brain, kidney, and lung tissues obtained at necropsy. Electron microscopy of infected SP-1K cells revealed the presence of virions having morphology and size resembling members of the Flaviviridae. Final identification as SLE virus was made by neutralization and immunofluorescence staining tests.

Key words: Flavivirus; Orcinus orca; Encephalitis; Marine mammal

Introduction

Case Report

A mature male killer whale (Orcinus orca), estimated to be 25 or more years old, presented with reduced appetite. Within 24 h, the animal became lethargic. The animal died in September, 1990, within 48 h after the onset of clinical signs, while under veterinary care. The necropsy was unremarkable, but the histopathology examination reported a non-suppurative meningoencephalitis.

Laboratory Studies

Virus isolation. Frozen samples of brain, lung, kidney and several other organs were sent to the ATCC Virology Laboratory as part of an ongoing screen for viruses of cetaceans. All tissues were thawed, mixed with sterile sand and tissue culture medium, and aseptically ground with a mortar and pestle. The suspensions were clarified by low-speed centrifugation (500 x g for 10 min) and the supernatants were used to
inoculate primary African Green Monkey kidney cells, SP-1K (a dolphin cell line, ATCC CCL 78) cells and bovine turbinate (ATCC CRL 1390) cells. Upon initial inoculation, bacterial contaminations were noted in all cultures. All first passage cultures were frozen, thawed and blind passaged on fresh cells in the presence of 100 μg/ml gentamycin sulfate. Possible CPE was noted in SP-1K cells inoculated with the brain, kidney and lung samples. These samples were passed through a 0.2-μm filter and a third passage was made on SP-1K cells. A definitive CPE was then observed from the brain, lung and kidney samples; cells became rounded and refractile and detached from the flask. No further attempts were made to isolate the agent from the bovine turbinate and primary monkey kidney cells.

The bacterial contaminants, although not identified, do not at this time appear related to the animal's death. Many of the necropsy samples received by ATCC for viral screening from cetaceans have bacterial or fungal contaminants. This is due in part to the cetacean's large size and insulating layer of blubber which make it impossible to quickly cool the animal after death. As a result, bacteria from the intestine and other non-sterile areas quickly multiply and spread throughout the peritoneal cavity.

**Electron microscopy.** When infected SP-1K cells showed distinct CPE, they were removed from the flask and fixed with 2% glutaraldehyde for two hours. Fixed cell suspensions were sent to the VA Virology Laboratory in West Haven, Connecticut by overnight mail. The cells were postfixed in osmium tetroxide and embedded in Spurr's resin as described previously (Hsiung, 1982). Thin sections were cut, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Fig. 1). For each of the three (brain, lung and kidney) SP-1K samples, numerous enveloped virus particles 40–50 nm in size were seen in the cytoplasm. Each particle had an electron-dense core 25 nm in diameter; virus particles budding from the plasma membrane were also observed (Fig. 1, inset). With the noted size and morphology, the isolate was tentatively identified as a member of the Flaviviridae family.

**Final identification.** A virus preparation propagated from the brain sample, having a titer of $10^{4.5}$ tissue culture infectious doses (TCID$_{50}$) was inactivated by exposure to 20% chloroform for two hours. A neutralization test on the same virus preparation was performed in SP-1K cells. Antisera to Japanese encephalitis virus (ATCC VR-1259AF) suppressed the appearance of CPE for 72 h, but CPE subsequently developed. In contrast, antisera to St. Louis encephalitis virus (ATCC VR-1265AF) completely neutralized the infectious virus through the entire 7 days incubation. In addition, acetone-fixed infected and non-infected SP-1K cells were sent to Dr. Karabatsos at the CDC Arbovirus Diseases Branch of the Division for Vector-Borne Infectious Disease, CDC in Fort Collins, CO, for confirmation. Using monoclonal SLE type-specific antibody (Roehrig et al., 1983), the three isolates were identified as St. Louis encephalitis virus by the immunofluorescent staining test (Nick Karabatsos, personal communication). It should be noted that, although two strains of SLE are available through the ATCC, neither has been propagated at the ATCC during the past four years that the marine mammal screening program has been active. Consequently, it is very unlikely that the three SLE isolates, from a single animal, were the result of cross-contamination from existing stocks.
Discussion

We have been unable to find any reference to SLE virus infecting marine mammals in the current literature (McLean and Bowen, 1980; Buck and Schroeder, 1990; Kennedy-Stospkopf, 1990; Monath, 1990). Since 1933, when the SLE virus was first isolated from a human brain, there have been many recognized epidemics of St. Louis encephalitis in the United States. The clinical cases associated with these outbreaks, and the intervening endemic periods, have been most frequent in California, Texas, Florida, Ohio, Illinois, and Indiana (Monath, 1990).

SLE is a member of the Japanese Encephalitis (JE) antigenic subgroup of the Flaviviridae (Wengler, 1991). Wild birds appear to be the primary (maintaining) vertebrate host, and the virus is transmitted by mosquitoes from infected viremic birds to susceptible birds. When conditions are suitable (large numbers of viremic birds and large numbers of mosquitoes), transmission to incidental hosts such as humans and horses occurs. Although several members of the JE subgroup are
pathogenic for humans, only the prototype JE strain is economically important as a disease of domestic animals (Fenner, 1987). SLE virus does not cause clinical disease in horses and other domestic mammals (Monath, 1990). Even in humans where SLE can cause severe encephalitis, many cases of SLE go undiagnosed or are subclinical (Monath, 1990).

In the present case, the killer whale apparently contracted SLE virus during a recognized SLE outbreak in Florida (CDC, 1990a,b). It is likely that, as with humans, killer whales are incidental hosts in the SLE cycle. Other cetaceans in the vicinity of the infected animal showed no illness consistent with SLE and were seronegative for SLE antibody (data not presented). The absence of reports associating illness or death of any marine mammal with SLE or other member of the Japanese encephalitis group further suggests that infections of marine mammals by infected mosquitoes are quite rare or, as in the case of many terrestrial mammals, most SLE infections are subclinical.

This report of an unexpected isolation of St. Louis Encephalitis virus from a cetacean demonstrates the importance of viral screening in animal species where the knowledge of viral pathogens is limited. The EM examination provided a rapid presumptive identification of an unknown agent which was confirmed by antibody neutralization and immunofluorescent staining tests. Studies are currently under way to identify two additional agents recently isolated in cell culture from other marine mammal specimens.

Acknowledgements

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Sea World of Florida Technical Contribution 9213-F.

References