Necropsy Report

Killer Whale (Orcinus-orca) Nami
Age 28 yrs — Port of Nagoya Aquarium, Japan

Name: Nami (female) (aka Nami-chan)

Species: Killer Whale (Orcinus orca)

Source: wild capture, 10-01-1985, Taiji Coast, Japan, age: est. 2yrs

Deceased: 7:24 pm, 01-14-2011, Port of Nagoya Aquarium, Japan, age: est. 28 yrs

Initial reported cause of death: (February, 2011) Fungal Bacterial Pneumonia, Stomach Ulcers, Myocardial Fibrosis. Note: 491 stones weighing 81.4 kilograms (179.5 lbs) were lodged in her stomach compartments.

Update October 22, 2011: Necropsy results were presented by a team of veterinarians at the Asian Zoo/ Wildlife Histopathology and Parasitology Conference, hosted by the Asian Society of Wildlife Pathology and Parasitology—

Diagnosis: A case of pulmonary zygomycosis with Cunninghamella bertholletiae in a killer whale (Orcinus orca). This case represents the first documentation of zygomycosis associated with C.bertholletiae in a marine mammal.

Discussion: Fungi may be primary or secondary pathogens that emerge during any chronic infectious process in cetaceans. Based on histopathology findings, possible causes of death include necrotizing pneumonia, pulmonary cavitations, and severe purulent bronchopneumonia with presumptive subsequent septicemia caused by opportunistic microflora.

See the full text of the necropsy report on the pages that follow.

The first stomach showing impaction caused by a large amount of ingested stones.

Notes: Prior to reforms of the Marine Mammal Protection Act (MMPA) in 1994, holders of marine mammals for public display in the U.S.A. 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.

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Case 3

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CASE HISTORY:
Signalment:
An adult female killer whale (Orcinus orca), 28 years old

Clinical history
The presented case had been moved to Port Nagoya Public Aquarium at the beginning of June in 2010 from another aquarium, where the whole had been maintained for over 20 years. The animal exhibited gradual loss of body weight over several months after its arrival. Signs of anorexia and gastrointestinal disorders first appeared at the beginning of October 2010. The animal developed severe anorexia in the middle of December 2010 and died on January 14, 2011.

Blood examination done on October 5, 2010 revealed an elevated total white blood cell count (WBC) (12,500/μl), fibrinogen level (344 mg/dl) and decreased alkaline phosphatase (ALP) (154 IU/l). Follow-up blood tests done 3 and 10 days later revealed the same results, with a decrease in the RBC count. Blood samples obtained on January 5, 2011 showed marked elevation of the WBC (21,250/μl), fibrinogen level (711 mg/dl), and aspartate aminotransferase (AST) (44 IU/l) and a decreased RBC count (306 x 10⁶/μl) and Hb level (9.5 g/dl). In addition, the ALP value demonstrated a continuing decrease (93 IU/l). Blood analysis 7 days later (January 12) showed anemia, a sudden decrease in WBC (7,100/μl) and ALP (82.1 IU/l) and an increase in AST (109 IU/l), ALT (26 IU/l), along with continuous elevation of fibrinogen (834 mg/dl) and LDH (945 IU/l).

Gross findings:
At necropsy, the body weight was 2,450 kg, and the body measured 589 cm in length. The animal was poorly nourished. One of the most striking gross findings was the marked enlargement of the stomachs; three stomachs contained a large number of palpable stones measuring 1 to 15 cm in diameter. The wall of the first stomach was markedly thinned, and contained 474 various-sized stones, with a total weight of 69.2 kg. The second stomach had 16 stones weighing 11.5 kg in all. The third stomach contained one stone weighing 0.7 kg (120 x 80 x 60 mm). The stones ranged in size from a few cm to 17 cm in diameter, and in shape from round to oval, with smooth surfaces. Multiple petechial hemorrhages with severe erosions were observed in the gastric mucosa of the second and third stomachs. The thoracic lymph nodes were markedly enlarged and edematous. Both lungs were enlarged and were firm in texture, with numerous nodules, especially in the upper half of the lungs. On cut surfaces, there were severe diffuse consolidations, along with multi-focal liquefactions, as well as cavitations measuring 3 to 5 cm in size in the lungs. The cavities had coalesced to each other to form larger cavities, and were usually filled with varying amounts of dark-colored, caseous material. In the heart, there was a large amount of pericardial fluid (hydropericardium) consisting of approximately 2,000 ml of clear, tan-colored fluid. Impression smears from the lungs revealed numerous bacteria and frequent non-septated
fungal hyphae. Other examined organs were within the normal limits.

**Histopathological Findings:**
In the lungs there was a severe degree of necrotizing pneumonia associated with suppurative bronchopneumonia along with frequent cavitations. The bronchi and alveoli were markedly dilated and packed with abundant purulent and mucopurulent exudates consisting of neutrophils, macrophages and cellular debris, as well as numerous bacteria colonies. Inside the pulmonary cavities, there were various degrees of lining zonal bacterial colonies embedded in the necrotic tissues, which were surrounded by numerous fungal hyphal elements. Under PAS and GMS staining, the hyphae were seen to be 30 to 40 μm in diameter, infrequently septated, thin-walled and tangled, and usually demonstrated a variable pattern of branching. Lymph nodes had severe lymphadenitis. The liver showed marked congestion, in addition to slight to moderate periportal lymphocytic infiltrations. The spleen showed severe congestion, sub-capsular multi-focal hemorrhages and moderate lymphoid depletion, in addition to diffuse extramedullary hematopoiesis represented by the presence of erythroblasts and megakaryocytes. Both kidneys showed slight congestion. In the stomach, the lining mucosa of the different gastric compartments showed multi-focal hemorrhages extending to ulcers in the third stomach, with frequent bacterial colonies seen on the surface of the ulcerating mucosa as well. The small and large intestines showed chronic enteritis with moderate to severe lympho-plasmacytic infiltrations in the lamina propria. **Immunohistochemistry:** The hyphal element in the lung lesions revealed strong and uniform positive reactions in the cell walls of the hyphae with Anti-Rhizomucor, but was negative for Anti-Aspergillus and Anti-candida albicans.

**Fungal cultures and identification of the fungus:** A zygomycosis, Cunninghamamella bertholletiae, was identified by morphological characteristics (Kwon-Chung and Bennett 1992) and finally confirmed by molecular techniques using the internal transcribed spacer (ITS) region of the ribosomal DNA (Kano et al. 2011). Comparative sequence analyses of the clinical isolate with the ITS region in GenBank showed that its queried sequence was 100% identical to C. bertholletiae (GenBank accession number FJ345351). **Microbiological analysis and identification of the isolated bacteria:** Bacteriology testing for aerobic and anaerobic microorganisms yielded Proteus mirabilis, Pseudomonas aeruginosa and Pseudomonas oryzihabitans in the lungs.

**Diagnosis:** A case of pulmonary zygomycosis with Cunninghamamella bertholletiae in a killer whale (Orcinus orca)

**Discussion:** Fungi may be primary or secondary pathogens that emerge during any chronic infectious process in cetaceans. Based on histopathology findings, possible causes of death include necrotizing pneumonia, pulmonary cavitations, and severe purulent bronchopneumonia with presumptive subsequent septicemia caused by opportunistic microflora. It is possible that septicemia and toxemia could cause general congestion and systemic multi-focal degenerative areas. Pulmonary cavitations and necrosis are suspected to be caused by Cunninghamamella bertholletiae. The present case may possibly be the first report of pulmonary mycotic infection associated with order Mucorales, Cunninghamamella bertholletiae sp. combined with severe suppurative bronchopneumonia which appeared to have been caused by Proteus mirabilis, Pseudomonas (P) aeruginosa and P. oryzihabitans.

The most outstanding pathological findings involving C. bertholletiae have been described as angioinvasion accompanying hemorrhages and necrosis. The primary site of zygomycoses infection usually involves the subcutaneous tissues and skeletal musculature; however, the
lungs, nasal sinus and alimentary tract are obvious entry portals for the fungus. Gastric ulcerations may have facilitated the invasion of the fungus as reported in a harbor porpoise.

Mycoses in cetaceans may be indicative of immunosuppression. Stressful conditions, including a large number of stones, ulcerations of the third stomach and transportation might be putative factors that facilitate mycosis. In the present case, there was a striking abnormality in the stomachs, in the form of a large amount of stones being present, especially in the first and second gastric compartments. A heavy load of stones could be a source of continuous mechanical injuries, physical pressure, hemorrhages, gastritis, and gastric ulcerations, as well as circulatory disturbances.

References:

Fig. 1 The first stomach showing impaction caused by a large amount of ingested stones.

Fig. 2 Lungs showing multi-focal cavity formation in the purulent consolidation with necrotic areas and abscesses.

Fig. 3 Fungi showing infrequently septated hyphae tangled with each other. GMS stain.