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Salmonella Newport Omphaloarteritis in a Stranded Killer Whale (*Orcinus orca*) Neonate

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ABSTRACT: *Salmonella enterica* serovar Newport (*Salmonella* Newport) was isolated from multiple tissues in a neonate killer whale (*Orcinus orca*) that stranded dead in 2005 along the central coast of California, USA. Necrotizing omphaloarteritis and omphalophlebitis was observed on histologic examination suggesting umbilical infection was the route of entry. Genetic analysis of skin samples indicated that the neonate had an offshore haplotype. Salmonellosis has rarely been identified in free-ranging marine mammals and the significance of *Salmonella* Newport infection to the health of free-ranging killer whales is currently unknown.

Key words: Killer whale neonate, omphaloarteritis, *Orcinus orca*, *Salmonella enterica* serovar Newport, salmonellosis.

Although many marine species are known to harbor *Salmonella enterica*, reports of *Salmonella*-associated disease in free-ranging cetaceans are rare (Minette, 1986). Foster et al. (1999) report isolating *Salmonella* (primarily a monophasic group B *Salmonella*) from 39 wild harbor porpoise (*Phocoena phocoena*) stranded on the coastline of Scotland, and hypothesized that in this species the bacterium might be an opportunistic invader as opposed to a primary pathogen. Jepson et al. (2000) diagnosed bronchopneumonia and septicemia caused by Group B *Salmonella* in two stranded harbor porpoise from the western Atlantic Ocean. On the west coast of North America, *Salmonella* has been isolated from sea otters (*Enhydra lutris nereis*; Smith et al., 2002) and pinnipeds, including northern elephant seals (*Mirounga angustirostris*; Stoddard et al., 2008), California sea lions (*Zalophus californianus*; Stoddard et al., 2008), northern fur seals (*Callorhinus ursinus*; Gilmartin et al., 1979), and harbor seals (*Phoca vitulina*; Thornton et al., 1998). There is a single report of *Salmonella*-associated septicemia in an adult female harbor porpoise from Washington, USA (Norman et al., 2004).

Just as little is known about *Salmonella* in free-ranging marine mammals, little is known about infectious diseases in killer whales (*Orcinus orca*). In a review, Gaydos et al. (2004) identified only 3 infectious agents reported from free-ranging killer whales and 13 from captive killer whales. This case report describes *Salmonella enterica* serovar Newport (*Salmonella* Newport) –associated bacteremia, omphaloarteritis, and phlebitis in a stranded killer whale neonate.

On December 7, 2005 the carcass of a 152.7-kg, female killer whale calf was discovered on Hollywood Beach, Ventura County, California, USA (34°09′50″N, 119°13′49″W). The carcass was in good nutritional and postmortem condition (postmortem condition code 2; Geraci and Lounsbury, 2005). The body was transported to the Santa Barbara Museum of Natural History, Santa Barbara, California, USA, and frozen pending gross necropsy. The whale had a straight total length (snout to tail notch) of 232 cm and

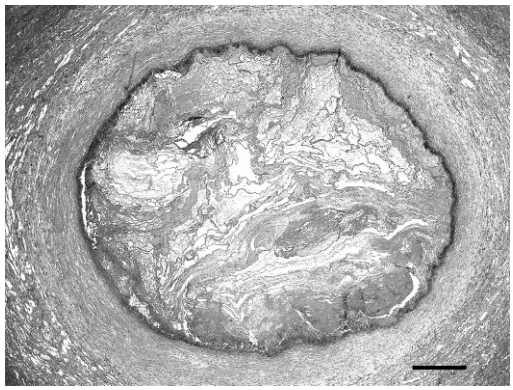

FIGURE 1. Omphaloarteritis of the umbilical artery in a killer whale neonate. The lumen of the artery is filled with fibrin and necrotic debris. H&E. Bar=1 mm.

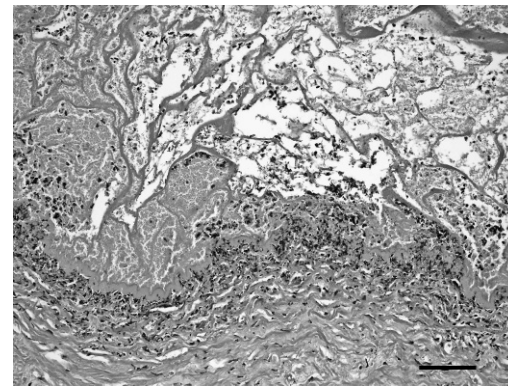

FIGURE 2. High-power photomicrograph of the inner layer of the umbilical artery with necrotizing omphaloarteritis. The endothelium is absent and internal elastic membrane partially obscured by necrotic debris and degenerate neutrophils.. H&E. Bar=100 μm.

an axillary girth of 113.5 cm. The skin along the ventrum and saddle patch was light yellow, and several thin circumferential folds (fetal folds) were noted in the skin along the thorax and trunk. The teeth had not erupted through the gingival mucosa and the umbilicus was closed. Based on the standard measurements and presence of fetal skin folds, the animal was determined to be a full-term neonate (Clark et al., 2000).

Significant gross necropsy findings were limited to the umbilical vessels, spleen, uterus, and lymph nodes. On the cut section, segmental areas of both the umbilical artery and umbilical vein were filled with thick yellow exudate. The left retropharyngeal lymph node was diffusely congested and edematous, and the spleen was mildly enlarged and diffusely congested. There was approximately 1.0–2.0 ml of clear red fluid within the lumen of the uterine horns. The lungs were diffusely aerated and sections floated in formalin. Intestinal contents were limited to a small amount of light yellow to brown thick fluid throughout the small intestine and colon with no evidence of meconium.

Multiple tissue sections were fixed in 10% neutral buffered formalin. Tissues were processed routinely, sectioned at 4 μm, and stained with hematoxylin and eosin. Sections of umbilical blood vessels, lymph nodes, and spleen also were stained with Brown and Brennen (Luna, 1968). On microscopic examination, the lumens of both the umbilical artery and vein contained large amounts of fibrin and necrotic debris (Fig. 1). The endothelium was completely denuded and the tunica intima and internal elastic membrane partially obscured by necrotic debris and degenerate neutrophils (Fig. 2). Small numbers of lymphocytes, plasma cells, and fewer neutrophils infiltrated the tunica media and surrounded the vasa vasorum. On Brown and Brennen staining, there were small numbers of Gram-negative short bacterial rods within the wall of the umbilical artery and vein. No bacteria were observed in sections of lymph node or spleen. Histologic lesions were not apparent in the other tissues examined, including the lungs, heart, liver, gastrointestinal tract, reproductive tract, skeletal muscle, skin, brain, spleen, or lymph nodes.

Tissue sections from the hilar and marginal lymph nodes, cerebellum, cerebrum, spleen, uterus, and umbilical artery were collected for microbiologic analysis. Aerobic bacterial culture and antimicrobial sensitivity testing was performed at the

Microbiology Laboratory, Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, California, USA and at the Animal Health Centre, British Columbia Ministry of Agriculture and Food, Abbotsford, British Columbia, Canada. Samples were cultured on a sheep blood agar plate, MacConkey agar plate, and trypticase soy broth, and incubated aerobically at 37 C under 5% CO_2. At both laboratories, all tissues sampled yielded medium-sized grey-white nonhemolytic colonies on the blood agar plate and colorless nonlactose fermenting colonies on MacConkey agar. The Gram-negative bacilli were identified as *Salmonella enterica* via standard biochemical identification. *Salmonella* antiserum testing showed a positive reaction to serogroup C2 for all isolates. Further serotype characterization of the isolate according to the O (somatic) and H (flagellar) antigens was performed at the National Veterinary Services Laboratory, Ames, Iowa, USA. The isolate was identified as *Salmonella enterica* serovar Newport (*Salmonella* Newport) and were sensitive to all antimicrobials tested including amikacin, amoxicillin/clavulanic acid, ampicillin, cefazolin, ceftizoxime, chloramphenicol, enrofloxacin, gentamicin, tetracycline, ticarcillin/clavulanic acid, and trimethoprim-sulfamethoxazole.

Fecal flotation and sedimentation failed to reveal parasites or ova. Polymerase chain reaction performed on pooled tissue samples was negative for *Brucella* spp., canine distemper virus, dolphin distemper virus, *Chlamydia* spp., influenza virus, *Mycoplasma* spp., and *Toxoplasma gondii*. Virus isolation using Vero and Mabin Dawby cells failed to yield cytopathic effect. Serum protein electrophoresis performed at Sea World, San Diego, California, USA yielded a spike in the beta globulin fraction and a small peak in the gamma fraction, suggesting that passive transfer of immunoglobulins had occurred and there was developing immune system function.

Three distinct killer whale ecotypes, called resident, transient, and offshore, are recognized in northeastern Pacific Ocean. Ecotypes are behaviorally, ecologically, genetically and morphologically distinct (Wiles, 2004). Genetic analysis of skin samples completed at the National Oceanographic and Atmospheric Administration, Southwest Fisheries Science Center, La Jolla, California, USA revealed that the stranded neonate had an offshore haplotype. Relatively little is known about the offshore killer whale ecotype because of infrequent sightings, but these whales are thought to remain predominately in deep offshore waters and feed primarily on fish (Jones, 2006).

Salmonella spp. have been isolated from the feces of marine birds and mammals along the coast of California, but their association with disease in these animals is not well understood (Thornton et al., 1998; Smith et al., 2002; Stoddard et al., 2008). In this killer whale, the isolation of *Salmonella* Newport in pure culture from multiple organs in the absence of significant inflammation suggests that acute bacteremia likely played a role in this animal's stranding and death. Bacterial umbilical infections are relatively common in stranded pinniped pups and septicemia is a frequent sequela (Colegrove et al., 2005). In this whale there is a good chance that umbilical infection was the source of infection. Very little is known about *Salmonella* in killer whales. There is a single report of *Salmonella* infection in a captive killer whale (Ridgway, 1979); however, to our knowledge, this is the first report of isolation of a *Salmonella* sp. from a free-ranging killer whale.

Over the last decade there has been a surge in multidrug-resistant (MDR) *Salmonella* Newport in humans, with isolates being routinely resistant to ampicillin, amoxicillin/clavulanic acid, cephalothin, cefoxitin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline and having either decreased susceptibility or resistance to extended-spectrum cephalo-

sporins, such as ceftriaxone (Varma et al., 2006). The emergence of MDR *Salmonella* Newport in humans has coincided with the emergence of MDR *Salmonella* Newport infections in cattle and several retrospective studies of *Salmonella* Newport infections in humans demonstrated that infections in residents of New England (Gupta et al., 2003) and Wisconsin (Karon et al., 2007) are associated with exposure to dairy farms and unpasteurized milk. Although California is one of the top 10 milk-producing states in the United States, the source of *Salmonella* Newport infection in a cetacean that primarily inhabits deep offshore waters is unknown. Unlike the surge in human and cattle cases of *Salmonella* Newport, this isolate from this killer whale was susceptible to all antimicrobials tested and was not associated with MDR *Salmonella* Newport of human or bovine origin. Interestingly, the one other reported case of *Salmonella* in a cetacean from the west coast of the United States also was identified as *Salmonella* Newport (Norman et al., 2004); however, antimicrobial susceptibility was not reported. The potential impact that *Salmonella* Newport could have on offshore, resident, or transient killer whale populations is unknown and bears further consideration. In order to learn more about the role that *Salmonella* plays in causing disease in killer whales and other marine mammals, it would be prudent for wildlife disease diagnosticians to continue to screen all stranded marine mammals for these pathogens and to conduct antimicrobial sensitivity testing on isolates.

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