

DETECTION OF *Brucella* spp ANTIBODIES IN POST MORTEM HEART BLOOD AND ANTEMORTEM SERUM OF KILLER WHALES (*Orcinus orca*) IN THE PACIFIC NORTHWEST

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ABSTRACT

Brucellosis is caused by a gram negative, intracellular coccobacilli with infections typically associated with exposure to carrier animals, contaminated meat or unpasteurized milk products, through cuts and abrasions, or inhalation of aerosols. The bacterium has a predilection for the reticuloendothelial (liver, spleen, lymph nodes and bone marrow), musculoskeletal and urogenital systems and less commonly affects gastrointestinal, cardiovascular and neurologic sites. There are six recognized species of *Brucella* which are variably host specific and exclusively infect terrestrial mammals. With the recent isolation and characterization of *Brucella* spp from marine mammals in 1994, there has been a proposal to expand the current six nomen species to either seven (*B. maris*) or eight (*B. pinnipediae* and *B. cetaceae*).

After initial serologic detection and isolation of *Brucella* spp from harbor seals (*Phoca vitulina*) within the Puget Sound, Washington State in 1997 and demonstration of antibody titers in harbor seals on the northeast coast of Vancouver Island and Georgia Strait, British Columbia in 2001, efforts were to expanded to investigate the prevalence and geographic distribution of antibody titers in stranded marine mammals within the Pacific Northwest. In particular, due to the potential impact of infection on fecundity and reproductive failure, as well as the declining population status of southern resident killer whales (*Orcinus orca*), efforts to serologically evaluate post mortem heart blood and serum from live killer whales were initiated.

Between 2000 and 2002, three killer whales stranded within the Pacific Northwest. Comprehensive necropsies were conducted on two of three animals and post mortem heart blood was collected from all three animals and forwarded to the Animal Disease Research Institute, Nepean, ON (Canada) for serology. As part of the diagnostic evaluation of a northern resident whale (A73) prior to transport from Seattle, Washington to Hanson Island, British Columbia, in

June, 2002, serum was also collected and submitted for analysis (Table 1). In all four cases, samples were evaluated by competitive ELISA and were positive. With L60, *Brucella* spp like colonizes were propagated *in vitro* and subsequently lost prior to phenotypic characterization.

The salient gross and microscopic findings are listed below (Table 1). Although post mortem change impeded microscopic assessment of tissue, there were no discernible lesions within the examined slides which could be directly attributed to brucellosis. Immunohistochemistry of select tissues was negative and polymerase chain reaction is currently being implemented and validated for marine mammal variant and universal *Brucellae* primers.

Similarly, initial field investigations in Europe and North America failed to reveal any consistent pathology associated with positive bacteriologic or serologic findings. However, in more recent strandings of multiple small cetacean species, subcutaneous abscessation, meningoencephalitis, orchitis, endometritis, abortion, and vertebral osteomyelitis have been reported. Efforts to intensify surveillance and post mortem examination of stranded free ranging killer whales as well as serologic investigation of captive killer whales may provide additional insights into the pathogenesis and potential impact of this bacteria in wild populations.

Table 1. Location and post mortem findings in *Brucella* spp seropositive killer whales (*Orcinus orca*) in the Pacific Northwest

Whale	State/Province	Site	Comments
J18, southern resident, 2000	British Columbia	Tsawwassen	Lateral flank, cutaneous abrasion with localized cellulitis and dermatitis with generalized septicemia due to <i>Edwardsiella tarda</i> , elevated contaminants, post mortem heart blood positive
CA189, transient, 2002	Washington	Dungenese spit	<i>Brucella</i> seropositive and <i>Brucella</i> -like organism isolated, but lost in culture, marked myocardial fibrosis, cutaneous abrasions overlying head, chronic gastritis, and nutritional myopathy
L 60, southern resident, 2002	Washington	Long Beach	<i>Brucella</i> antibodies detected in post mortem heart blood, no significant gross or microscopic lesions
A73, northern resident, 2002	Washington	Seattle	Live capture and transport, seropositive for <i>Brucella</i> antibodies, possible inborn error of metabolism and cachexia