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CRYPTOCOCCOSIS DUE TO *CRYPTOCOCCUS GATTII* IN STRANDED MARINE MAMMALS IN THE EASTERN NORTH PACIFIC

Cryptococcosis is a fungal infection of humans and a variety of animal species including cats, dogs, horses, cattle, exotic pets and others. The two main fungal species are *Cryptococcus neoformans* and *C gatti*, which are dimorphic, basiodiomycetes with a predilection for the nervous and respiratory systems. Infections are typically associated with environmental contamination and generally derived from inhalation or penetrating wounds. Neither pathogen is considered zoonotic or contagious. *C neoformans* tend to infect immunocompromised hosts, are ubiquitous, saprophytic, and associated with soil, pigeon guano, and organic detritus; whereas, *C gattii* has historically been limited to tropic and subtropic regions, often coinciding with the distribution of Eucalyptus trees, and infections are recognized in immunocompetent individuals.

In 1999, the first multispecies outbreak of cryptococcosis due to *C gattii* was recognized on Vancouver Island, British Columbia (BC), with subsequent dispersal to the lower mainland, then Washington State in 2005, Oregon in 2004 and California in 2008. The index wildlife case in BC was believed to be an adult harbor porpoise (*Phocoena phocoena*) stranded in Nanaimo, BC.

In the northeastern Pacific, there are several regional stranding response programs which recover and necropsy dead marine mammals. On review of stranding records, between 1997 and 2016 there were 42 cetaceans that stranded in BC and Washington State with cryptococcosis, including 26 harbour porpoises (*P phocoena*), 14 Dall's porpoises (*Phocoenoides dalli*) and 2 Pacific white sided dolphins (*Lagenorhynchus obliquidens*). Infected animals typically presented with gross lesions of severe emaciation, lymphadenopathy and pneumonia, often with variably sized gelatinous masses throughout the tissues. Histopathology revealed florid intralesional yeast with prominent clear capsules, granulomatous pneumonia, lymphadenitis with necrosis, cavitation, and hemorrhage, encephalitis and multisystemic fungemia. Incidental findings included verminous pneumonia, gastric nematodiasis, and biliary trematodiasis. In 1 pregnant female, there was vertical transmission from the dam to the developing fetus.

Diagnosis of cryptococcosis is usually based on yeast morphology in histologic slides or by cytologic preparations. Fungal isolation and speciation are required for a definitive diagnosis. In addition to routine histopathology and bacteriology, special culture for *C gattii* was undertaken with stranded cetaceans with gross lesions and isolates were forwarded to an outside reference laboratory for multilocus sequence typing (MLST). In BC, 90-95% of *C gattii* isolates from human, veterinary, and environmental samples were VGIIa and 5-10% were VGIIb. Whole genome sequence advanced genotyping techniques to further distinguish clonal isolates. Single nucleotide polymorphism analysis has revealed the diversity between VGIIa, VGIIb, and VIIc, that were not previously detected by MLST and has revealed much greater diversity in VGIIb than in VGIIa and VGIIc, suggestive of more recent emergence.

The source of the multispecies *C gattii* outbreak has not yet been fully resolved. The fungus has been recovered from a variety of tree species on Vancouver Island, including Douglas fir (*Pseudotsuga menziesii*), red alder (*AInus rubra*), Western red cedar (*Thuja plicata*), Grand fir (*Abies grandis*) and Garry oak (*Quercus garryana*). Risk factors associated with human, cat and dog infections include hiking, soil disturbance, logging and urban expansion, which may have resulted in aerosolization of infective stages (propagules) and exposure. These anthropogenic activities, coupled with recent increases in ambient temperature (climate change) may surpassed a threshold for activation, propagation and possible sexual recombination of dormant stages of the fungus. An alternative scenario includes dispersal of infective

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stages from tropical climes (Australasia) via aerosolization and transport by prevailing trans-Pacific air mass flow (estimated 3-5 day duration to transit the Pacific). Based on spatiotemporal extent of the outbreak and MLST, cases of cryptococcosis in marine mammals tended to cluster around terrestrial *C gattii* hotspots.

Between 2012 and 2013, there was a decline in the number of reported human and veterinary cases of *C gattii* infection in British Columbia. The last reported case of cryptococcosis in a cetacean was in a harbor porpoise in 2016. Efforts are ongoing to recover and survey stranded marine mammals for cryptococcosis. Ironically, in 2012 a novel fungal infection, mucormycosis was reported in dead beach cast harbor seals (*Phoca vitulina*), harbor porpoises and a southern resident killer whale (*Orcinus orca*). The primary purpose of a marine mammal stranding response program is the early detection of pathogens, which may impact individual and population health, establish spatial and temporal data baselines, and improve our understanding of infectious disease ecology. Ongoing support through the Canadian Wildlife Health Cooperative, regional stranding response programs, an informed public and the Department of Fisheries and Oceans, has improved this response.

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