DOI: 10.7589/2015-11-299

Journal of Wildlife Diseases, 52(3), 2016, pp. 677–681

Cryptococcus gattii Type VGIIa Infection in Harbor Seals (*Phoca vitulina*) in British Columbia, Canada

Justin F. Rosenberg,[1,5] Martin Haulena,[1] Linda M. N. Hoang,[2,3] Muhammad Morshed,[2,3] Erin Zabek,[4] and Stephen A. Raverty[4] [1]Vancouver Aquarium Marine Science Center, 845 Avison Way, Vancouver, British Columbia V6G 3E2, Canada; [2]British Columbia Center for Disease Control Public Health Lab, 655 W 12th Ave., Vancouver, British Columbia, V5Z 4R4 Canada; [3]Department of Pathology and Laboratory Medicine, University of British Columbia, 2211 Wesbrook Mall, Vancouver, British Columbia V6T 2B5, Canada; [4]Animal Health Centre, BC Ministry of Agriculture, 1767 Angus Campbell Road, Abbotsford British Columbia V3G 2M3, Canada; [5]Corresponding author (email: rosenbergdvm@gmail.com)

ABSTRACT: Cryptococcosis has been reported in marine mammals in the northeastern Pacific with increasing frequency in the last 15 yr. Although a variety of cetaceans have been diagnosed with cryptococcosis, *Cryptococcus gattii* has not been reported in pinnipeds. We document *C. gattii* VGIIa in a harbor seal (*Phoca vitulina*) pup and in an unrelated adult. Both animals were presented to Vancouver Aquarium's Marine Mammal Rescue Centre (VAMMRC) with generalized weakness, dehydration, respiratory compromise, minimally responsive mentation, and suboptimal body condition. Necropsy and histopathology findings were consistent in both animals and featured generalized lymphadenopathy, bronchopneumonia, and meningoencephalitis with intralesional yeast and fungemia. Cryptococcal serum antigen titers were $\geq$1,024 in both animals. Fungal culture of lung and lymph nodes confirmed *C. gattii*. Exposure was likely via inhalation prior to presentation to VAMMRC, and *C. gattii* infection was the proximate cause of death. This report expands the range of susceptible host species as *C. gattii* continues to emerge as a pathogen of concern in marine mammals in the northeastern Pacific.

Key words: Cryptococcosis, *Cryptococcus gattii*, harbor seal, *Phoca vitulina*, pinniped, systemic mycosis.

Since the late 1990s, cryptococcosis has been an emerging zoonotic disease in southwestern British Columbia, Canada (Fyfe et al. 2008). Subsequent to initial detection in humans and veterinary case material on Vancouver Island, British Columbia, the incidence of *Cryptococcus gattii* infection in western Canada is now among the highest world-wide (Datta et al. 2009). *Cryptococcus gattii* has been documented in a variety of wildlife and domestic species including dogs (*Canis familiaris*), cats (*Felis catus*), horses (*Equus caballus*), goats (*Capra hircus*), bottlenose dolphin (*Tursiops truncatus*), spinner dolphin (*Stenella longirostris*), Dall's porpoise (*Phocoenoides dalli*), and harbor porpoise (*Phocoena phocoena)* (Miller et al. 2002; Stephen et al. 2002; Lester et al. 2004, 2011; Rotstein et al. 2010; Duncan et al. 2011; Norman et al. 2011; Huckabone et al. 2015). There is one report of a California sea lion (*Zalophus californianus*) with *Cryptococcus albidus* infection; however, despite intensive surveillance and recovery efforts, no cases of *C. gattii* have been identified in pinnipeds (Mcleland et al. 2012).

Cryptococcus gattii is one of over 37 members of the genus *Cryptococcus* within the order Tremellomycetes (Lester et al. 2011). It is a basidiomycetous yeast that, unlike *Cryptococcus neoformans*, is considered a pathogen of immunocompetent individuals (Sorrell 2001). Until recently, *C. gattii* was believed to be limited to the tropical and subtropical regions, paralleling the distribution of eucalyptus trees (Sorrell 2001); its emergence in temperate regions has led to numerous collaborative investigations into the extent of environmental contamination, disease transmission, and potential risk of human and animal exposure (Kidd et al. 2007). Exposure to *C. gattii* can have different clinical manifestations depending on the infected species, route of exposure, inoculum dose, and stage of pathogenesis of infection, but is commonly associated with respiratory and neurologic disease with more-generalized tissue involvement in a subset of animals

(Chen et al. 2014). Typical lesions observed with *C. gattii* in humans include meningoencephalitis, pulmonary cryptococcomas, and cryptococcemia (Chen et al. 2012). In cetaceans, *C. gattii* infection typically presents as bronchopneumonia (Stephen et al. 2002).

On 16 September 2014, an approximately 3-wk-old female harbor seal (*Phoca vitulina*) was recovered from a beach in Roberts Creek along the Strait of Georgia, British Columbia, Canada (49°25′12″N, 123°37′12″W). The animal was initially assessed and stabilized and then transported to Vancouver Aquarium's Marine Mammal Rescue Centre, Vancouver, British Columbia. The seal weighed 9 kg, was dehydrated, malnourished, and had multiple puncture wounds on the hind flippers. Initial complete blood count and serum chemistry identified a significant leukocytosis (31.2×10^9/L), neutrophilia (25.6×10^9/L) with left shift (1.2×10^9/L bands), transient hyperglycemia (16.1 mmol/L) associated with prior dextrose administration, and age-related changes consistent with published reference ranges (Greig et al. 2010). She was treated supportively with oral fluids (Revibe®, Zoetis Canada, Kirkland, Quebec, Canada) via gastric tube, subcutaneous fluids (Lactated Ringer's, B. Braun Medical Inc., Scarborough, Ontario, Canada), and was started on oral ciprofloxacin (10 mg/kg, 250 mg tablets, Mylan Pharmaceuticals ULC, Etobicoke, Ontario, Canada) and carprofen (2 mg/kg, 25 mg tablets, Zoetis Canada). She remained quiet and rousable but died on the third day post admission. A large volume of mucohemorrhagic discharge was evident from the nares at the time of death.

On 2 April 2015, a second, unrelated animal stranded on a beach in Parksville, British Columbia (49°19′48″N, 124°16′48″W). This adult male animal weighed 90 kg, was dehydrated, underconditioned, and minimally responsive to external stimuli. Complete blood count and serum chemistry obtained the day the animal was found identified a moderate leukocytosis (17.3×10^9/L) and azotemia (blood urea nitrogen [BUN] 15.8 mmol/L; creatinine 107 μmol/L). Repeated blood work showed a persistent leukocytosis (16.1×10^9/L) with mildly improved azotemia (BUN 13.4 mmol/L; creatinine 75 μmol/L). He was initially treated with subcutaneous ceftiofur (7 mg/kg, 200 mg/mL, Excede 200, Zoetis Canada) and enrofloxacin (5 mg/kg, 50 mg/mL, Baytril®, Bayer Inc., Toronto, Ontario, Canada) in addition to intramuscular dexamethasone (0.25 mg/kg, 5 mg/mL, Dexamethasone5, Vetoquinol, Lavaltrie, Quebec, Canada). The animal passed a large volume of melena, so he was treated with intramuscular duplocillin (1 mL/10 kg, 300,000 IU/mL, Inervet Canada LTD, Whitby, Ontario, Canada). He was treated supportively with subcutaneous fluids (Lactated Ringers, B. Braun Medical) overnight. The following morning, whole body radiographs disclosed patchy pulmonary consolidation, but ultrasound did not detect thoracic effusion. The animal succumbed during a seizure on the second day post admission.

Necropsy and histopathology were consistent in both animals and revealed granulomatous lymphadenitis, meningoencephalitis, bronchopneumonia, and urinary cystitis (Fig. 1). Florid extracellular yeast morphologically consistent with *Cryptococcus* spp. were identified in associated inflammatory infiltrates in lymph nodes, brain, lung, and the urinary bladder (Fig. 2). The peripheral vasculature had prominent fungemia. Punctate cryptococcomas were observed in the pancreas, salivary gland, tracheal submucosa, and thyroid gland. There were no significant lesions in other tissues examined.

Fresh lung tissue and lymph nodes were submitted for fungal culture on Sabouraud dextrose agar with gentamicin (Oxoid Company, Nepean, Ontario, Canada) media and Columbia blood agar with 5% sheep blood (Oxoid). An isolate of *Cryptococcus* sp. from each animal was forwarded to the British Columbia Center for Disease Control Public Health Laboratory (Vancouver, British Columbia, Canada) for specific identification and genotyping. *Cryptococcus* genotyping was performed using restriction fragment length polymorphism PCR methodology as previously described (Kidd et al. 2004, 2005). Briefly, the *Ura5* gene fragment was amplified and

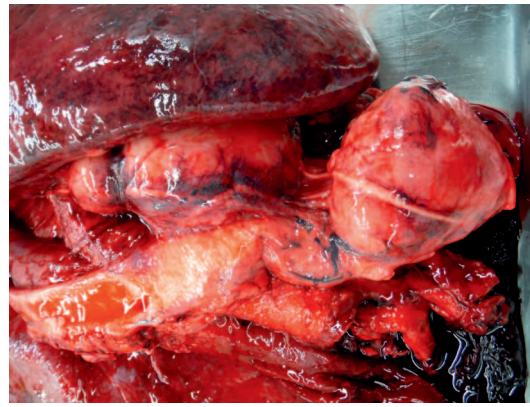

FIGURE 1. Mediastinal and thoracic inlet lymphadenomegaly in an adult, male harbor seal (*Phoca vitulina*) diagnosed with disseminated *Cryptococcus gattii* VGIIa infection in British Columbia, Canada, 2015.

digested using two sets of restriction enzymes (Set A: *Hha* and *Sau*96I and Set B: *Hha*, *BsrG* I, and *Dde* I). *Cryptococcus gattii* genotypes were determined based on band patterns. The genotyping studies confirmed these isolates as *C. gattii* VGIIa. Molecular diagnostics to screen for additional pathogens were performed and proved negative for Apicomplexa, *Brucella* spp., and canine distemper virus with methods previously described (Barrett et al. 1985; Casans et al. 2001; Gibson et al. 2011). Serum antigen titers for *Cryptococcus* spp. were ≥1,024 for both animals (CrAg® Lateral Flow Assay, Imprimis Pharmaceuticals, San Diego, California, USA). The necropsy findings, histopathology, fungal culture, and serum antibodies confirm that these animals died from disseminated *C. gattii* VGIIa infection. Based on a review of case records, these are believed to be the first cases of *C. gattii* in harbor seals or any pinniped species.

With cryptococcosis, clinical findings in affected animals can often vary based on host species and route of exposure (Chen et al. 2014). In terrestrial species, *C. gattii* may be introduced via inhalation, retrograde invasion via the cribriform plate, ingestion, and direct inoculation by a penetrating wound (Kidd et al. 2007; Pennisi et al. 2013). Based on the distribution and extent of lesions in these

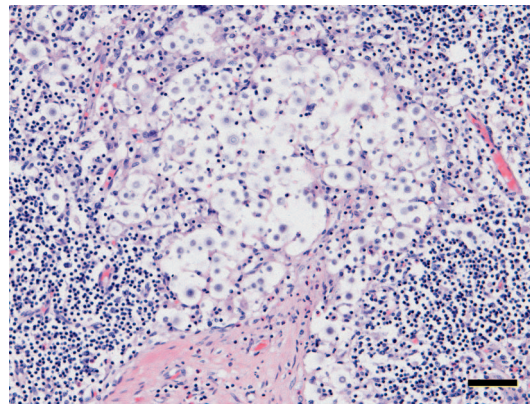

FIGURE 2. Microphotograph of an enlarged tracheobronchial lymph node from a harbor seal (*Phoca vitulina*) with paratrabecular expansion and replacement of the cortex by nodular accumulation of yeast (*Cryptococcus gattii*), British Columbia, Canada, 2015. There is a paucity of inflammatory infiltrate in this lesion. H&E stain. Bar=20 μm. Microphotograph captured according to the protocol described by Marty (2007).

harbor seals, the route of exposure was likely via inhalation, and initial infection was presumably in the wild. There were no apparent pre-existing lesions which may have predisposed the animals to infection.

In utero transmission of *C. gattii* has been documented in a female harbor porpoise (Norman et al. 2011). Although infection of the pup in this case was likely incurred postpartum, the possibility of vertical transmission cannot be entirely discounted based on the age and severity of the lesions. The contribution of insufficient colostrum consumption or other disorders in predisposing this animal to infection is unknown. There has been an association of *C. gattii* in a Dall's porpoise (*Phocoenoides dalli*), with identical strains found in terrestrial animals, suggesting a common environmental source of exposure (Huckabone et al. 2015).

The diagnosis of cryptococcosis in these two cases expands the number of susceptible host species and suggests that *C. gattii* continues to pose a threat to human and animal populations. Fungal culture is recommended in suspected cases and empiric antifungal therapy may be indicated. Ongoing surveillance is recommended to further understand the implications of cryptococcosis for pinniped population health within the northeastern Pacific.

The authors thank all of the staff and volunteers at the Vancouver Aquarium's Marine Mammal Rescue Centre for their dedication and hard work on this patient and all of the other animals in their care. The staff of the Animal Health Center (Abbotsford, British Columbia, Canada) is also recognized for their assistance with the necropsies.

LITERATURE CITED

Barrett T, Shrimpton S, Russell S. 1985. Nucleotide sequence of the entire protein coding region of canine distemper virus polymerase associated (P) protein mRNA. *Virus Res* 3:367–372.

Casans MC, Queipo-Ortuno MI, Rodriguez-Torres A, Orduna A, Colmenero JD, Morata P. 2001. Specificity of a polymerase chain reaction assay of a target sequence on the 31-kilodalton *Brucella* antigen DNA used to diagnose human brucellosis. *Eur J Clin Microbiol* 20:127–131.

Chen SC, Meyer W, Sorrell TC. 2014. *Cryptococcus gattii* infections. *Clin Microbiol Rev* 27:980–1024.

Chen SC, Slavin MA, Heath CH, Playford EG, Byth K, Marriott D, Kidd SE, Bak N, Currie B, Hajkowicz K, et al. 2012. Clinical manifestations of *Cryptococcus gattii* infection: Determinants of neurological sequelae and death. *Clin Infect Dis* 55:789–798.

Datta K, Bartlett KH, Baer R, Brynes E, Galanis E, Heitman J, Hoang L, Leslie MJ, MacDougall L, Magill SS, et al. 2009. Spread of *Cryptococcus gattii* into Pacific Northwest region of the United States. *Emerg Infect Dis* 15:1185–1191.

Duncan C, Bartlett KH, Lester S, Bobsien B, Campbell J, Stephen C, Raverty S. 2011. Surveillance for *Cryptococcus gattii* in horses of Vancouver Island, British Columbia, Canada. *Med Mycol* 49:734–738.

Fyfe M, MacDougall L, Romney M, Starr M, Pearce M, Mak S, Mithani S, Kibsey P. 2008. *Cryptococcus gattii* infections on Vancouver Island, British Columbia, Canada: Emergence of a tropical fungus in a temperate environment. *Can Comm Dis Rep* 34:1–12.

Gibson AK, Raverty S, Lambourn DM, Huggins J, Magargal SL, Grigg ME. 2011. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. *PLoS Negl Trop Dis* 5:e1142.

Greig DJ, Gulland FMD, Rios CA, Hall AJ. 2010. Hematology and serum chemistry in stranded and wild-caught harbor seals in central California: Reference intervals, predictors of survival, and parameters affecting blood variables. *J Wildl Dis* 46:1172–1184.

Huckabone SE, Gulland FMD, Johnson SM, Colegrove KM, Dodd EM, Pappagianis D, Dunkin RC, Casper D, Carlson EL, Sykes JE, et al. 2015. Coccidioidomycosis and other systemic mycoses of marine mammals stranding along the central California, USA coast: 1998–2012. *J Wildl Dis* 51:295–308.

Kidd SE, Chow Y, Mak S, Bach PJ, Chen H, Hingston AO, Kronstad JW, Bartlett KH. 2007. Characterization of environmental sources of the human and animal pathogen *Cryptococcus gattii* in British Columbia, Canada, and the Pacific Northwest of the United States. *Appl Environ Microb* 73:1433–1443.

Kidd SE, Guo H, Bartlett KH, Xu J, Kronstad JW. 2005. Comparative gene genealogies indicate that two clonal lineages of *Cryptococcus gattii* in British Columbia resemble strains from other geographical areas. *Eukaryot Cell* 4:1629–1638.

Kidd SE, Hagen F, Tscharke RL, Huynh M, Bartlett KH, Fyfe M, MacDougall L, Boekhout T, Kwon-Chung KJ, Meyer W. 2004. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (BC, Canada). *Proc Natl Acad Sci U S A* 101:17258–17263.

Lester SJ, Kowalewich NJ, Bartlett KH, Krockenberger MB, Fairfax TM, Malik R. 2004. Clinicopathologic features of an unusual outbreak of cryptococcosis in dogs, cats, ferrets, and a bird: 38 cases (January to July 2003). *J Am Vet Med Assoc* 225:1716–1722.

Lester SJ, Malik R, Bartlett KH, Duncan CG. 2011. Cryptococcosis: Update and emergence of *Cryptococcus gattii*. *Vet Clin Pathol* 40:4–17.

Marty GD. 2007. Blank-field correction for achieving a uniform white background in brightfield digital photomicrographs. *Biotechniques* 42:716–720.

Miller WG, Padhye AA, van Bonn W, Jensen E, Brandt ME, Ridgway SH. 2002. Cryptococcosis in a bottlenose dolphin (*Tursiops truncates*) caused by *Cryptococcus neoformans* var. *gattii*. *J Clin Micro* 40:721–724.

Mcleland S, Duncan C, Spraker T, Wheller E, Lockhard SR, Gulland F. 2012. *Cryptococcus albidus* infection in a California sea lion (*Zalophus californianus*). *J Wildl Dis* 48:1030–1034.

Norman SA, Raverty S, Zabek E, Etheridge S, Ford JKB, Hoang LMN, Morshed M. 2011. Maternal-fetal transmission of *Cryptococcus gattii* in harbor porpoise. *Emerg Infect Dis* 17:304–305.

Pennisi MG, Hartmann K, Lloret A, Ferrer L, Addie D, Belák S, Boucraut-Baralon C, Egberink H, Frymus T, Gruffydd-Jones T, et al. 2013. Cryptoccosis in cats: ABCD guidelines on prevention and management. *J Feline Med Surg* 15:611–618.

Rotstein DS, West K, Levine G, Lockhart SR, Raverty S, Morshed MG, Rowles T. 2010. *Cryptococcus gattii* VGI in a spinner dolphin (*Stenella longirostris*) from Hawaii. *J Zoo Wildl Med* 41:181–183.

Sorrell TC. 2001. *Cryptococcus neoformans* variety *gattii*. *Med Mycol* 39:155–168.

Stephen C, Lester S, Black W, Fyfe M, Raverty S. 2002. Multispecies outbreak of cryptococcosis on southern Vancouver Island, British Columbia. *Can Vet J* 43: 792–794.

Submitted for publication 4 November 2015.
Accepted 8 February 2016.