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Locally Acquired Disseminated Histoplasmosis in a Northern Sea Otter (*Enhydra lutris kenyoni*) in Alaska, USA

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ABSTRACT: Histoplasmosis of local origin has not been reported in humans or wildlife in Alaska, and the disease has never been reported in a free-ranging marine mammal. In 2005 a northern sea otter (*Enhydra lutris kenyoni*) was found on Kodiak Island, Alaska, at 57° latitude north, far outside the known distribution of *Histoplasma capsulatum.* The animal died of disseminated histoplasmosis. Microorganisms consistent with *Histoplasma* sp. were observed on histopathology, and *H. capsulatum* was identified by PCR and sequencing. We suggest migratory seabirds or aerosol transmission through prevailing winds may have resulted in transmission to the sea otter.

Key words: Alaska, *Enhydra lutris*, *Histoplasma capsulatum*, northern sea otter, seabirds.

Sea otters (*Enhydra lutris kenyoni*) occur in near shore coastal waters along the North Pacific Rim from the Kuril Islands of Russia, through the Aleutian Islands of Alaska, and down the Pacific Northwest coast of North America to California. Since 2002 the US Fish and Wildlife Service (USFWS) in Alaska has been conducting necropsies on otters for a baseline health and disease study. A 1-yr-old female sea otter was reported dead on 4 March 2005 on Kodiak Island, Alaska (57°43′33″N, 152°30′59″W), and shipped to the USFWS laboratory in Anchorage, Alaska, for necropsy. Standard necropsy protocols were followed, and tissues were processed for histopathology. Aerobic cultures were performed on the spleen and gastrointestinal (GI) tract (Brownstein et al. 2011), and serum was tested by immunofluorescent antibody test for protozoans including *Toxoplasma gondii*, *Sarcocystis neospora,* and *Neospora caninu*m (Miller et al. 2011). DNA was extracted from frozen spleen, and extracted DNA was used as a template for PCR using pan-fungal primers to amplify the internal transcribed spacer region (ITS1-5.8S-ITS-2) of the ribosomal RNA gene, followed by sequencing and sequence analysis as previously described (Eshar et al. 2010). Total RNA was extracted from frozen lung and lymph node, and heminested PCR performed with universal morbillivirus primers and PDV-specific primer for phosphoprotein gene as previously described (Goldstein et al. 2009).

Gross findings included poor body condition, massive hepatosplenomegaly, generalized lymphadenopathy (Fig. 1), and marked thymic atrophy. There were mucinous exudates in the trachea and bronchi and lungs were edematous. Incidental findings included laryngitis, gingivitis, pharyngitis, ulcers in the pyloric region of the stomach, and small numbers of *Corynosoma* sp. attached to the intestinal lining with no evidence of transmural reaction. The lymphadenopathy and hepatosplenomegaly were due to massive infiltration of histiocytic macrophages packed with fungal microorganisms. These infected histiocytes were present in the red pulp in the spleen, medullary regions of lymph nodes, sinusoids of the liver, bone marrow, glomerular tufts, intravascular monocytes, lamina propria of the tonsil, tongue, and nasopharynx and expanded the alveolar and interlobular septa in the lung. In the spleen there were also areas of coagulation necrosis, extramedullary hematopoiesis, and depleted lymphoid follicles. Liver hepatocytes were compressed by large numbers of sinusoidal histiocytes replete with microorganisms. Intracellular

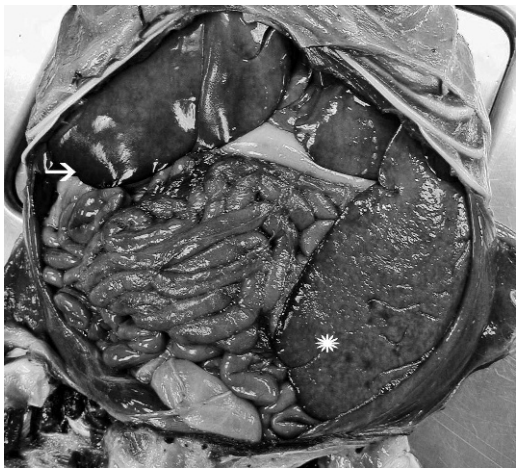

FIGURE 1. Histoplasmosis in an Alaskan sea otter (*Enhydra lutris*): massive hepatosplenomegaly. The head of the animal is toward the top of the photo. The arrow indicates the liver; the starburst indicates the spleen.

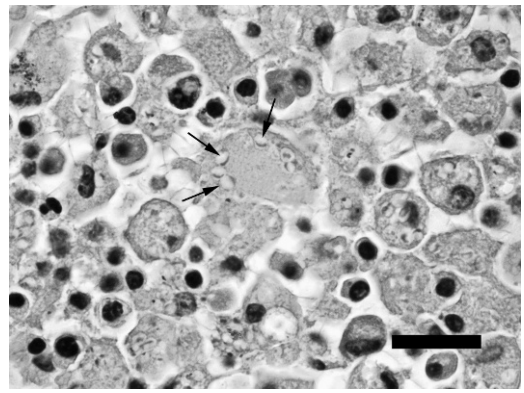

FIGURE 2. Histoplasmosis in an Alaskan sea otter (*Enhydra lutris*): Photomicrograph of the intrahistiocytic microorganisms within macrophages in the spleen (100×). The microorganisms are approximately 3–4 μm in diameter, have a capsule, clear vacuole, and lightly basophilic, round to crescent-shaped microorganisms. The microorganisms are arranged in a horseshoe-shaped pattern along the periphery of one of the Kupffer cells. Bar = 20 μm.

organisms were also present in hepatocytes and adrenocortical cells.

The microorganisms were 2.6–4 μm in diameter with a round, clear vacuole containing round to oval to crescent, lightly basophilic refractile body. The microorganisms either packed and expanded the cytoplasm of the cells or lined the periphery of the cytoplasm (Fig. 2). These microorganisms stained with Grocott's Methenamine Silver and periodic acid-Schiff and did not stain with acid-fast or Gram stains.

The differentials for these microorganisms included *Histoplasma* sp., *Leishmania* sp., *Pneumocystis* spp., and nonencapsulated *Cryptococcus* sp. Because routine aerobic cultures and fungal cultures were negative, the microorganism was characterized molecularly. The ITS region from the otter had 99.8% sequence identity with *Ajellomyces capsulatus* ATCC 38904 (GenBank accession no. AF322378), with less than 87% sequence identity to the next closest match among other fungal species, *Emmonsia (Ajellomyces) crescens*. *Ajellomyces capsulatus* is the anamorph form of *H. capsulatum*.

Aerobic cultures were negative on the spleen. Beta-hemolytic *Streptococci*, nonenteric sp., *Corynebacterium* sp., and *Streptococcus viridans* were found in the GI tract. The animal also had tonsillitis and laryngitis with intralesional fibrin and gram-positive cocci with culture of a β-hemolytic *Streptococcus* sp., nonenteric, bacterial species and *Corynebacterium* sp. from the larynx; all are common oral flora. These findings along with the premature thymic atrophy suggest the animal was immunocompromised. Sea otters in Kodiak have been exposed to phocine distemper virus (PDV; Goldstein et al. 2009), a morbillivirus linked to immunosuppresion. However, lung and lymph node tissue from this animal were negative for PDV and canine distemper virus by PCR. The animal was also serologically negative for *Toxoplasma gondii*, *Sarcocystis neurona*, and *Neospora caninum* by IFA on postmortem serum.

This animal had a severe disseminated infection with *H. capsulatum* as confirmed by morphology, PCR, and DNA sequencing. This is the first reported case of *H. capsulatum* originating in Alaska in humans or wildlife (Castrodale and Ritter

2001). There is, however, a suggestion that *H. capsulatum* was present in Alaska 1,600 yr ago, as a mummy from this time period had lesions consistent with the infection (Zimmerman and Smith 1975). Although *H. capsulatum* has been diagnosed in a wide variety of wild and captive animals (Burek 2001), and has been detected around the world (Mochi and Edwards 1952), there are no other reports of infection in wild marine mammals and only three reports in captive marine mammals (Wilson et al. 1974; Jensen et al. 1998; Morita et al. 2001).

How or why this wild Alaskan sea otter developed fulminant, systemic histoplasmosis is perplexing, and a source of exposure is difficult to explain because this fungus is associated with damp humid conditions between 45° latitude north and 45° latitude south worldwide. In the US, histoplasmosis infection is endemic in the Mississippi and Ohio River valleys and their tributaries (Grayston et al. 1955). This sea otter was found at 57° latitude north, far outside the known distribution of *H. capsulatum* in a maritime climate of mild winters and moist, cool summers. Because sea otters do not migrate, the infection must have been locally acquired.

Histoplasmosis is generally associated with exposure to habitats where birds and bats congregate in large numbers such as chicken coops, blackbird roosts, pigeon lofts, and bat roosting sites (Burek 2001). The fungus grows best in the upper 2.5–5.0 cm of moist soil with high nitrogen content, ideally when enriched with guano for a minimum of 3 yr. Transmission occurs through aerosols of contaminated soil, which remains infectious for years. *Histoplasma* can survive for long periods of freeze-defrost cycles and periods of deep freezing (Fischer and Barnum 1960).

Bats can become infected with *H. capsulatum,* transmitting the microorganism in their droppings. Little brown bats (*Myotis lucifugus*) are the only *Chiroptera* that occur on Kodiak (Parker et al. 1997) with estimates suggesting thousands are present in the summer. They are distributed in the forested regions of the archipelago, utilizing buildings and trees, and it is possible they are in areas where sea otters haul out.

Although birds are not infected with *H. capsulatum*, they can shed it in and carry it on their wings, feet, and beaks. One possible source is the tens of thousands of colonially nesting seabirds in the Kodiak archipelago. *Histoplasma capsulatum* was reported in the soil at a gull colony site in Michigan (Southern 1986). Unlike the bats, seabirds migrate large distances, in the winter going as far as California from their breeding grounds in Alaska (Hatch et al. 2011). Within 20 km of the infected otter's location there are approximately 20,000 Black-legged Kittiwakes (*Rissa tridactyla*) and 20,000 Tufted Puffins (*Fratercula cirrhata*; Sowls et al. 1978) where huge amounts of guano are excreted from cliff nesting sites close to otter habitat.

As an alternative to migratory birds as a source for transmission, this case could represent a new exposure due to airborne transmission of fungal spores on prevailing winds from distant sources such as Russia or Asia (Griffin 2007) as has been discussed with the introduction of *Cryptococcus gatii* in the Pacific Northwest (Duncan et al. 2006; Kidd et al. 2007a, b). However, it would be odd to have the disease occur in just one sea otter if this were the case. More research is needed to understand why histoplasmosis was found in this sea otter on Kodiak Island and what the implications are for this threatened species and the humans. We suggest testing the soil and guano around the pigeon and bat roosts, and soil around the largest seabird nesting colonies in Kodiak. We also suggest that disease work be conducted on bats in Alaska, since these mammals are an excellent terrestrial indicator of several major diseases and potential changes due to climate change (Jones et al. 2009).

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