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**Toxoplasmosis in a Hawaiian Monk Seal (Monachus schauinslandi)**

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**ABSTRACT:** Toxoplasma gondii infection in marine mammals is intriguing and indicative of contamination of the ocean environment with oocysts. T. gondii was identified in a Hawaiian monk seal (Monachus schauinslandi) that had visceral and cerebral lesions. Tachyzoites were found in the lymph nodes, spleen, diaphragm, heart, adrenal glands, and brain. A few tissue cysts were found in sections of the cerebrum. The diagnosis was confirmed serologically, by immunohistochemical staining with T. gondii-specific polyclonal rabbit serum, and by the detection of T. gondii DNA. The genotype was determined to be type III by restriction fragment length polymorphisms of the Sag2 gene. This is the first report of T. gondii infection in a Hawaiian monk seal.

Toxoplasma gondii infections have been reported in many nonthermic animals, including several species of marine mammals (Dubey and Beattie, 1988; Resendes et al., 2002; Dubey et al., 2003). Recently, concerns have been raised that T. gondii may be a cause of mortality in sea otters, which are an endangered species in U.S. waters (Thomas and Cole, 1996; Lindsay et al., 2001; Miller, Gardner, Kreuder et al., 2002). Viable T. gondii was isolated from 15 of 67 (Cole et al., 2000) and 24 of 75 (Miller, Gardner, Packham et al., 2002) dead sea otters, indicating that T. gondii infection is common in this animal. It has been suggested that sea otters become infected with T. gondii oocysts in the sea from freshwater coastal runoff (Miller, Gardner, Kreuder et al., 2002). The Hawaiian monk seal (Monachus schauinslandi) is an endangered pinniped in U.S. waters and its population has declined since 1950. We report fatal T. gondii infection in this host for the first time. An adult male Hawaiian monk seal (RK07) was found dead in the surf zone on 22 January 2004 at Otsuka beach on the east side of the island of Kauai, near the town of Kapaa. The animal was covered with ice, and a necropsy examination was performed 15 hr later. This animal was in good nutritional condition as evidenced by normal blubber thickness. The submandibular, prescapular, and tracheobronchial lymph nodes were enlarged and darker than normal. The cranial lung lobes were edematous, glistening, and gelatinous bilaterally. The stomach contained 2 L of fluid, and the bile duct was enlarged. Helminths were found in the trachea and stomach. Tissue specimens were fixed in 10% formalin and submitted to the Armed Forces Institute of Pathology, Washington, D.C., for histologic examination. Tissues were processed routinely, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Tissues were also forwarded to the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, for Sag2 gene examination. Deparaffinized sections of tissues were stained immunohistochemically with T. gondii and Neospora caninum polyclonal sera as described (Lindsay and Dubey, 1989; Dubey et al., 2001). In addition, sections were allowed to react with anti-BAG1 polyclonal rabbit antibody specific for Bradyzoites (McAllister et al., 1996). A sample of frozen blood was thawed, centrifuged, and the serum was tested for antibodies to T. gondii using the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987). For molecular confirmation, DNA was extracted from the lymph nodes of the monk seal using a DNAeasy tissue kit (Qiagen, Valencia, California) according to the manufacturer’s instructions. The DNA sample was tested for the amplification of the T. gondii-specific Sag2 fragments (Howe et al., 1997), along with positive and negative controls. The PCR products were electrophoresed in a 2% agarose gel with molecular weight standards. Genotype was determined by restriction fragment length polymorphisms (RFLP) of the Sag2 gene (Howe et al., 1997).

Prominent lesions were observed in multiple tissues (lymph nodes, spleen, adrenal glands, diaphragm, and brain), and characterized by necrosis with variable numbers of extracellular and intracellular protosozial tachyzoites. The most severe lesions were within the lymph nodes (Fig.
Lesions and *T. gondii* in sections of lymph nodes (A–C) and cerebrum (D, E) of the Hawaiian monk seal stained with hematoxylin and eosin (A, B, D) or with *T. gondii* antibodies (C, E). (A) Note a large central area of necrosis (arrow). (B) Numerous extracellular tachyzoites (arrows). (C) Numerous *T. gondii* tachyzoites (all black areas). (D) Note 6 tissue cysts (arrows) and a group of tachyzoites (arrowheads). (E) Note that tachyzoites (arrows) stained immunohistochemically are larger in size than tachyzoites stained with hematoxylin and eosin in (B).

The medulla of the lymph nodes contained large areas of lytic necrosis, hemorrhage, and numerous extracellular protozoal tachyzoites. Scattered throughout the cortex of the lymph nodes were numerous intracellular tachyzoites. The spleen was similarly affected with large areas of necrosis containing both extracellular and intracellular tachyzoites. Within the adrenal cortex there were multifocal, variably sized areas of lytic necrosis with hemorrhage and a few tachyzoites. Less affected areas of the adrenal cortex contained few intracellular tachyzoites. There were few random, multifocal areas of necrosis and rare intracellular tachyzoites within the diaphragm. The heart had a single aggregate of intracellular tachyzoites. The brain had few scattered glial nodules, a few extracellular and intracellular tachyzoites, and few small tissue cysts (Fig. 1C, D).

Other histologic findings included minimal multifocal lymphocytic menigitis with gliosis and hemorrhage, moderate diffuse pulmonary congestion with alveolar edema, minimal multifocal epicardial nodular granulomatous steatitis with edema, mild focal histiocytic and neutrophilic gastritis with intralesional metazoan parasite, moderate multifocal testicular interstitial hemorrhage with minimal subacute epididymitis, and multiple intraluminal cestode larvae within the small intestine. In addition, there was moderate autolysis in several tissues with significant postmortem bacterial overgrowth.

Protozoa in sections of lymph nodes, spleen, adrenal glands, lung, and the cerebrum reacted positively with *T. gondii* polyclonal antibodies (Fig. 1C, E) and not with *N. caninum* antibodies. Tissue cysts in brain reacted with BAG 1 antibodies. Antibodies to *T. gondii* were found in a 1:100 dilution of the serum even though the sample was hemolyzed. Amplifications of the specific SAG2 products were noticed from the DNA obtained from the lymph node of the monk seal as well as the positive control. The isolate was found to have the type III genotype on RFLP.

The character of lesions suggests that the seal acquired *T. gondii* infection recently, most likely through ingestion of oocysts. There was severe necrosis of lymph nodes with numerous tachyzoites. The cerebral...
lesions were localized, acute in nature, with only small tissue cysts present. In animals fed oocysts, initial lesions occur in the intestines and mesenteric lymph nodes, and some hosts can die before infection is established in the brain (Dubey and Beattie, 1988). Neural lesions are formed 2–3 wk after infection and initially consist of small areas of focal necrosis. With the passage of time, glial nodules are formed and tachyzoites begin to disappear from neural lesions.

The diagnosis of toxoplasmosis was confirmed in this animal immunohistochemically, by detection of antibodies, and DNA specific for T. gondii. The genotype of the isolate was found to be type III. All 48 isolates of T. gondii from sea otters genotyped thus far were either type II or a new genotype (Cole et al., 2000; Miller et al., 2004). The present case is the first indication of the presence of type III genotype in a marine mammal. It is also the first amplification of T. gondii DNA directly from the marine host. Previous data have been based on T. gondii isolates obtained by bioassay in cell culture or laboratory animals.

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LITERATURE CITED


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Trematodes Associated with Mangrove Habitat in Puerto Rican Salt Marshes

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ABSTRACT: Batillaria minima is a common snail in the coastal estuaries of Puerto Rico. This snail is host to a variety of trematodes, the most common being Cercaria caribaea XXXI, a microphallid species that uses crabs as second intermediate hosts. The prevalence of infection was higher (7.1%) near mangroves than on mudflats away from mangroves (1.4%). Similarly, there was a significant positive association between the proportion of a site covered with mangroves and the prevalence of the microphallid. The association between mangroves and higher trematode prevalence is most likely because birds use mangroves as perch sites and this results in local transmission to snails.

A visit to the Center for Disease Control website on travelers’ health underscores how parasite transmission can vary greatly from place to place, depending on environmental factors and the distribution of infectious stages. In the present study, we investigated whether habitat (mangrove vs. mudflat) affected the prevalence of trematodes in snails. Transmission of trematodes to snails should be highest in habitats that support many final hosts (Hoff, 1941; Cort et al., 1960; Robson and Williams, 1970; Sousa and Groszholz, 1991; Sapp and Esch, 1994; Smith, 2001; Kube et al., 2002; Skirnisson et al., 2004). For example, infections in some snail species are more prevalent near the littoral zone where final hosts are more abundant (Sapp and Esch, 1994; Esch et al., 1997). Recently, Skirnisson et al. (2004) related spatial heterogeneity in the prevalence of larval trematodes infecting Hydrobia ventrosa to both biotic and abiotic variables; they assumed that these factors influenced attractiveness of the habitats for definitive hosts, which ultimately produced the observed spatial heterogeneity in infections in first intermediate hosts. Smith (2001) found that bird droppings were highest near dead mangroves, where birds perched, compared to mudflats. In California, however, direct associations occur between birds and trematodes

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