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Source: Journal of Wildlife Diseases, 35(3) : 565-568

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-35.3.565

SHORT COMMUNICATIONS

Journal of Wildlife Diseases, 35(3), 1999, pp. 565–568

Hepatic Carcinoma with Spleen Metastasis in a California Sea Lion from the Gulf of California

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ABSTRACT: A primary hepatic carcinoma with a neuroendocrine pattern was detected in an adult female California sea lion (*Zalophus californianus*) found dead on Granito Island in the Gulf of California (Mexico) in January 1996. At necropsy, several light yellow nodules of different sizes were observed on the entire surface of the liver and spleen. Microscopic examination of these nodules using routine haematoxylin-eosin stain, revealed cubic, polyhedral and pleomorphic cells with three to four bizarre mitotic figures per field (40×). An immunohistochemistry test revealed a positive reaction of indirect immunoperoxide to cytokeratin (CK2). This is the first known case of a primary hepatic carcinoma in free-ranging California sea lions from Mexican waters.

Key words: California sea lion, case report, hepatic carcinoma, immunohistochemistry, neoplasm, pinnipeds, *Zalophus californianus*.

California sea lions (*Zalophus californianus*) are considered to be the most abundant and widely distributed pinniped species in Mexico (Aurioles-Gamboa et al., 1983). Recently, their population size in the Gulf of California was estimated at 30,000 individuals during the breeding season, with 2 to 3% annual rate of increase (Aurioles-Gamboa and Zavala, 1994).

Little is known about the morbidity and mortality of free-ranging California sea lions in Mexican waters; they have leptospirosis (C. R. Godínez, pers. comm.), parasitic pneumonia and gastrointestinal parasites (Aurioles and Sinsel, 1988), and heavy metal accumulation in body tissues (Becerril and Cuspineira, 1995). A recent mortality event in the northern part of the Gulf of California during January 1995 affected sea birds and marine mammals, including 51 California sea lions (Vidal and Gallo-Reynoso, 1996). Governmental authorities hypothesized biological- and human-induced causes. PCB's, organochlorine compounds, and heavy metal residues in the water and in some of the analyzed tissues have been reported (PROFEPA, 1995). Some of the environmental pollutants identified are considered potentially carcinogenic (Nunn et al., 1996).

Herein we describe a case of hepatic carcinoma with spleen metastasis in a California sea lion from the Gulf of California. This is the first report of this neoplasm in this species in Mexico.

In January 1996, the carcass of an adult female California sea lion was found on the west beach of Granito Island (29°33′N and 113°32′W) in the Gulf of California (Mexico). At necropsy, tissue samples of lung, mediastinic lymph nodes, liver, and spleen were obtained for histopathological examination. Samples were fixed in neutral buffered 10% formaldehyde at pH 7.4, embedded in paraffin, sectioned at 6 μm, and stained with haematoxylin-eosin (Luna, 1968). Liver and spleen tissues were subjected to an immunohistochemistry test done by the avidin-biotin-peroxidase complex method according to manufacturer's directions (Dako Corporation, Santa Barbara, California, USA), to ascer-

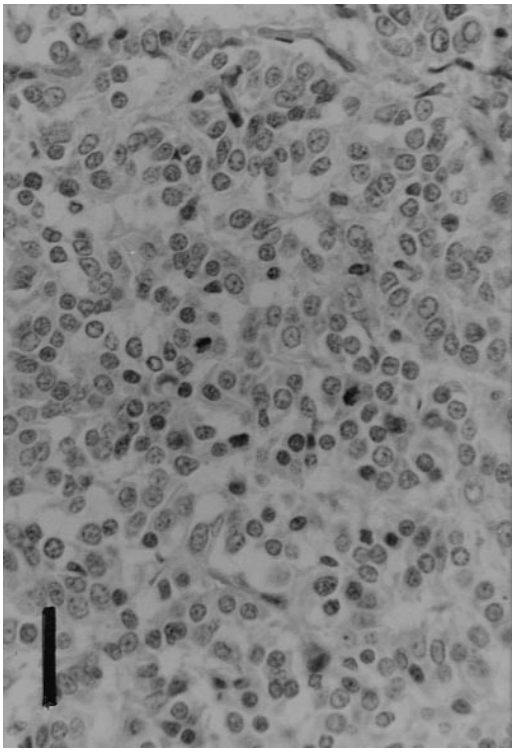

FIGURE 1. Hepatic carcinoma in liver tissue of a California sea lion from the Gulf of California showing cord arrangement of polyhedral cells and bizarre mitotic figures (arrows). H&E. Scale bar = 30 μm.

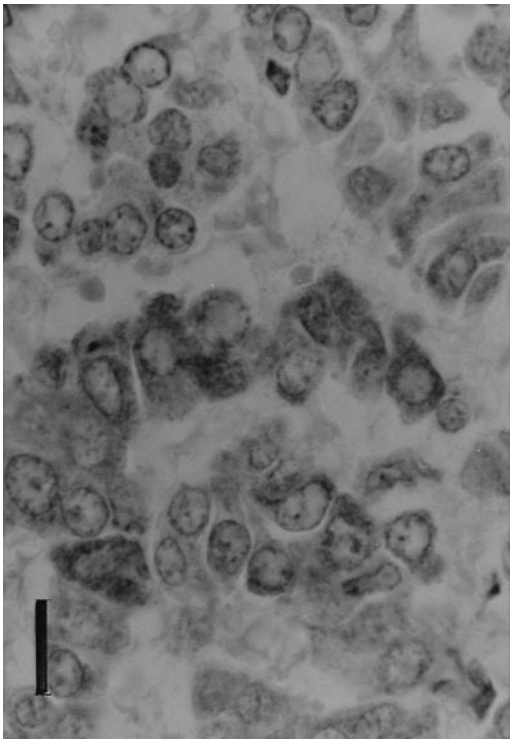

FIGURE 2. Hepatic carcinoma in liver tissue of a California sea lion from the Gulf of California showing positive immunostaining to cytokeratin. PAP. Scale bar = 12 μm.

tain the origin of the neoplastic tissues. Cytokeratin (CK2), epithelial membrane antigen (AME), and polyclonal embryonic carcinoma antigen (ACEp) antibodies serum (Dako Corporation) were used for this test.

The 166 cm long adult female California sea lion was emaciated, with only 3 mm of blubber thickness. Diffuse severe anasarca was found in the ventral abdominal region. The abdominal cavity contained approximately 500 ml of blood-tinged peritoneal fluid. The liver and spleen were severely congested and enlarged; both covered with multiple light yellow round nodules varying from 0.1 to 4 cm of diameter with several coalescent zones. Most nodules were umbilicated and had a dark center. When cut, they were white, firm and well defined with a slightly red center.

Histologic evaluation of liver and spleen revealed multiple areas of variable size with neoplastic cells arranged in cords, nests, and groups. Most nodules presented a necrotic center, with a sparse inflammatory reaction. Surrounding these areas, a thin layer of fibrous connective tissue issued trabeculae towards the interior of the affected tissue. Neoplastic cells were cubic, polyhedral, or pleomorphic in shape with moderate to abundant cytoplasm (Fig. 1). The nuclei were round and oval with fine granular chromatin and prominent nucleoli. Three to four bizarre mitotic figures per field (40×) were observed. The hepatocytes surrounding the neoplastic tissue were atrophied. A positive indirect immunoperoxide reaction to cytokeratin (CK2) (Fig. 2) along with a negative reaction to epithelial membrane antigen (AME) and polyclonal embryonic carci-

noma antigen (ACEp) were observed with immunohistochemistry. The final diagnosis was primary hepatic carcinoma with neuroendocrine pattern, and metastasis to the spleen.

The neoplasm was determined to be of hepatic origin, based on a positive reaction to cytokeratin (CK2) during the immunohistochemistry test (Rosai, 1989), and on the histological pattern which showed typical cords, groups, and nests of pleomorphic cells with anisonucleosis and bizarre mitotic figures (Popp, 1990). Hepatocellular carcinomas have been identified in several domestic species (Patnaik et al., 1980). They usually vary in size from small round lesions to large diffuse masses with a light tan to yellow color (Popp, 1990). Metastasis to abdominal tissues has been reported (Carlton and McGavin, 1995), with cells progressively invading the adjacent hepatic tissue (Popp, 1990) and then disseminating via the circulatory system to the lungs, heart, spleen, kidney, brain, and ovaries (Patnaik et al., 1980). The histologic appearance of hepatic carcinomas varies depending on the degree of differentiation and cell arrangement (Trigo et al., 1982). More differentiated neoplasms resemble normal liver while others form cords which appear as thickened trabeculae (Popp, 1990).

The suggested etiologies of neoplasms in captive and free-ranging pinnipeds include hormonal influences (Popp, 1990), viruses (Maxie, 1993) and chemical agents (Howard et al., 1983). Although a causal relationship between the development of neoplasms and pollutants in free-ranging marine organisms has not yet been proven (Nunn et al., 1996), some chemical factors have been suggested as causal agents for cancer in these animals (Griner, 1971). Two similar cases of hepatic metastatic adenocarcinoma observed in two California sea lions, found in the same geographic location and time, considered environmental carcinogen exposure as a possible cause (Brown et al., 1980). A wide range of potentially carcinogenic compounds and heavy metal pollutants like selenium, aluminum, and titanium (Britt and Howard, 1986) as well as polychlorinated biphenyl compounds (PCB's) and dichlorodiphenyltrichloroethane (DDT) (Nunn et al., 1996) have been found in ocean waters. If metabolized, organochlorinated compounds may lead to damage such as cellular membrane changes, lipid peroxidation, DNA modification, and enzyme inactivation (Livingstone, 1991), all of which contribute to the carcinogenic process (Nunn et al., 1996).

The Gulf of California receives continental discharge from various industries found mainly in its northern regions (PROFEPA, 1995). Because of marine water currents, these discharges are moved towards the central and midriff regions which is where the most important reproductive sea lion rookeries are found (Zavala, 1993). Although we report only one case of hepatic carcinoma in a single California sea lion, there may be public health implications that should be considered in terms of bioaccumulation of chemicals in the marine environment (Nunn et al., 1996). Further research must be directed towards the study of environmental pollutants and their effects in marine mammals. Assessment of genetic toxicity in pinniped tissues may allow monitoring some aspects of the status of environmental biopollutants.

The Armada de México and CONACyT, grant 1122PB, supported this work. All sampling was done by permission from the Instituto Nacional de Ecología; permit numbers DOO.-700-(2) 01104 and DOO.-700-(2).-1997.

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Received for publication 4 December 1997.