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Primary Pleural Squamous Cell Carcinoma in a Free-ranging River Otter (*Lontra canadensis*)

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ABSTRACT: An adult female North American river otter (*Lontra canadensis*) presented with multiple intrathoracic masses identified histologically as squamous cell carcinoma. Immunohistochemical staining patterns for high- molecular-weight keratin, p40, p63, calretinin, and TTF-1, along with the gross and histologic findings, indicated a primary pleural squamous cell carcinoma as the most likely diagnosis.

Neoplasia is rare in wildlife populations, with exceptions, such as urogenital carcinoma in California sea lions (*Zalophus californianus*), fibropapilloma in various sea turtle species, and devil facial tumor in Tasmanian devils (*Sarcophilus harrisii*; MacAloose and Newton 2009). Few neoplasms have been reported in mustelids; they include lymphosarcoma in domestic ferrets (*Mustela putorius*; Reed-Smith 2008) and limited reports of various neoplasms in sea otters *(Enhydra lutris),* with very low (1–2%) prevalence (Newman and Smith 2006). Reports of neoplasia in captive Eurasian river otters (*Lutra lutra*) are sparse, and include hepatocellular carcinoma, lymphosarcoma, malignant seminoma, malignant ovarian teratoma, cholangiocellular carcinoma, leiomyoma, pheochromocytoma, and differentiated basal cell carcinoma (Weber and Mecklenburg 2000; Bae et al. 2007). Dalton et al. (1997) reported successful surgical excision of a squamous cell carcinoma (SCC) from the tongue of an Asian small-clawed otter (*Aonyx cinerea*).

Primary pleural squamous cell carcinomas (PPSCC) have not been described in animals. They are rarely reported in humans and often pose a diagnostic challenge (Lin et al. 2013). Neoplasia may arise in areas of chronic inflammation, and in humans these tumors arise after a long latent period (11–47 yr) following chronic empyema, thoracic fistula, or pneumothorax (Rüttner and Heinzl 1997; Jeon et al. 2017). As PPSCC arise from the pleura, it is most likely that thoracic mesothelial cells are the cell type of origin.

A lethargic and emaciated free-ranging adult female river otter (*Lontra canadensis*) was found near a water treatment plant in Tiburon, California (37°52′25"N, 122°27′24"W), on 6 November 2017. It was caught and brought to a local veterinarian, who observed multiple masses in the dorsal thorax on radiology. After subcutaneous administration of 100 mL lactated Ringer's solution, and once daily, subcutaneous enrofloxacin (5 mg/kg, 2.5% Baytril®, Bayer AG), the animal developed sudden respiratory arrest and died. It was submitted for postmortem examination to The Marine Mammal Centre (TMMC), Sausalito, California, USA.

At necropsy, numerous white, multinodular and firm sessile masses up to 4 cm in diameter were distributed throughout the parietal pleura of the thoracic wall and diaphragm, with most centered around the thoracic inlet (Fig. 1A). The left lung lobe had one small mass on the pleural surface, and several thoracic lymph nodes (sternal and mediastinal) contained masses similar in consistency and color as the pleural masses. The other lung lobes were unaffected and no masses were found elsewhere in the body. The gastric mucosa had multiple mucosal ulcers (approximately 0.5-mm diameter) covered by a coagulum of blood, and parts of the jejunum contained dark red to black pasty contents (melena). Based on the gross lesions, the main differential diagnoses were neoplasia (mesothelioma, squamous cell carcinoma, or pul-

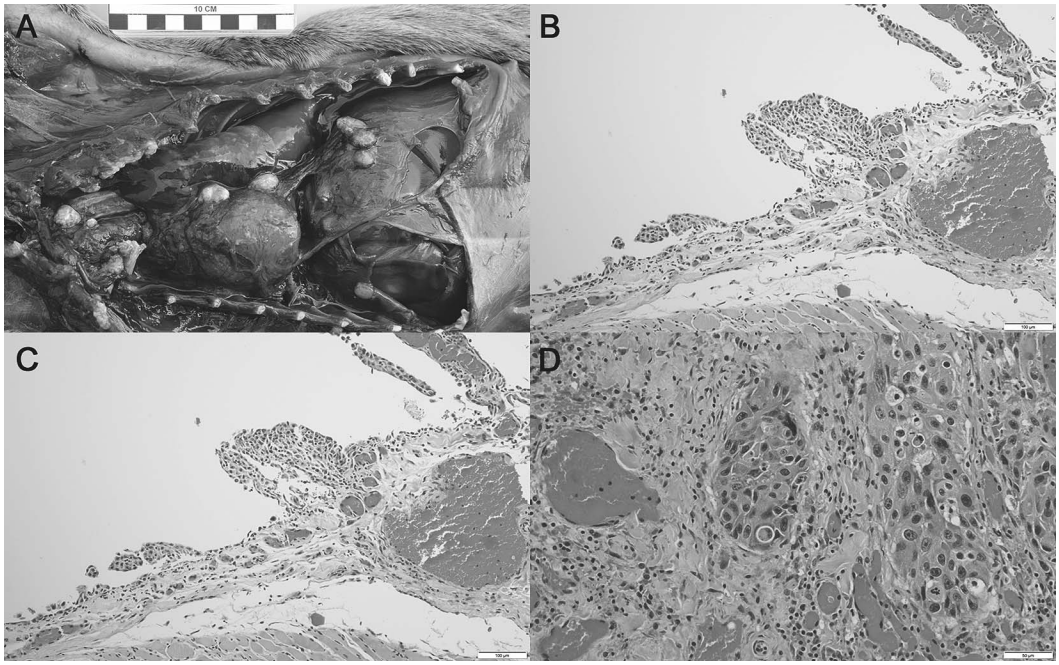

FIGURE 1. North American River otter (*Lontra canadensis*) gross and histopathology of thoracic primary pleural squamous cell carcinoma. (A) Thorax with sternum and sternal ribs excised to show multiple white nodules on the diaphragm, pleura, and mediastinum with sternal and mediastinal lymphadenomegaly. (B–D) Histologic appearance of thoracic masses. (B) Intercostal muscle with proliferative papillary projections of mesothelium. Scale bar = 500 μm. (C) Carcinoma in situ within the mesothelium. Scale bar = 100 μm. (D) Clusters of neoplastic cells within lymphatics. Scale bar = 50 μm.

monary bronchoalveolar carcinoma) or granulomatous inflammation (systemic mycosis such as *Coccidioides immitis* infection). Immunohistochemical staining was performed to distinguish between the three major neoplastic differentials with the use of anti–high-molecular-weight keratin (HMWK), anti-p40 and anti-p63 (both squamous cell nuclear markers), anti-calretinin (usually positive in mesotheliomas), and anti-thyroid transcription factor-1 (TTF-1), a marker for pulmonary cell origin usually absent in pulmonary SCC and mesothelioma (Weiss 2014; Caswell and Williams 2016).

Formalin-fixed tissue samples were embedded in paraffin wax and 3–5-μm sections were stained with hematoxylin and eosin (H&E). Immunohistochemical staining was performed on a Dako Omnis immunostainer (Agilent, Santa Clara, California, USA) with the use of primary antibodies anti-TTF-1 (Leica Biosystems Inc., Concord, Ontario, Canada, NCL-TTF-1, 1/200); anti-calretinin (Thermo Fisher Scientific, Waltham, Massachusetts, USA, 18-0211, 1/200); anti-HMWK (mouse monoclonal clone β34E14, Dako Canada Inc., Mississauga, Ontario, Canada, GA05161-2); anti-p40 (Biocare Medical, Markham, Ontario, Canada, BC-ACI3066C, 1/100) and anti-p63 (Leica Biosystems, NCL-p63, 1/400). All sections were subjected to 30 min of high-pH (9.0) antigen retrieval solution (Dako Canada, S236884-2). Dako Omnis instrument parameters used were as follows: anti-TTF-1: H20X20; anti-p63:H30-10M-30; anti-p40: H30-10M-30; β34E12: H25-10M-20, and anti-calretinin: H20X20. Staining was performed by Calgary Laboratory Services, Immunohistochemistry Laboratory (Calgary, Alberta, Canada).

Microscopically, sections of intercostal muscle with parietal pleura showed gradual transition from normal pleural mesothelium to proliferative papillary projections. Focally,

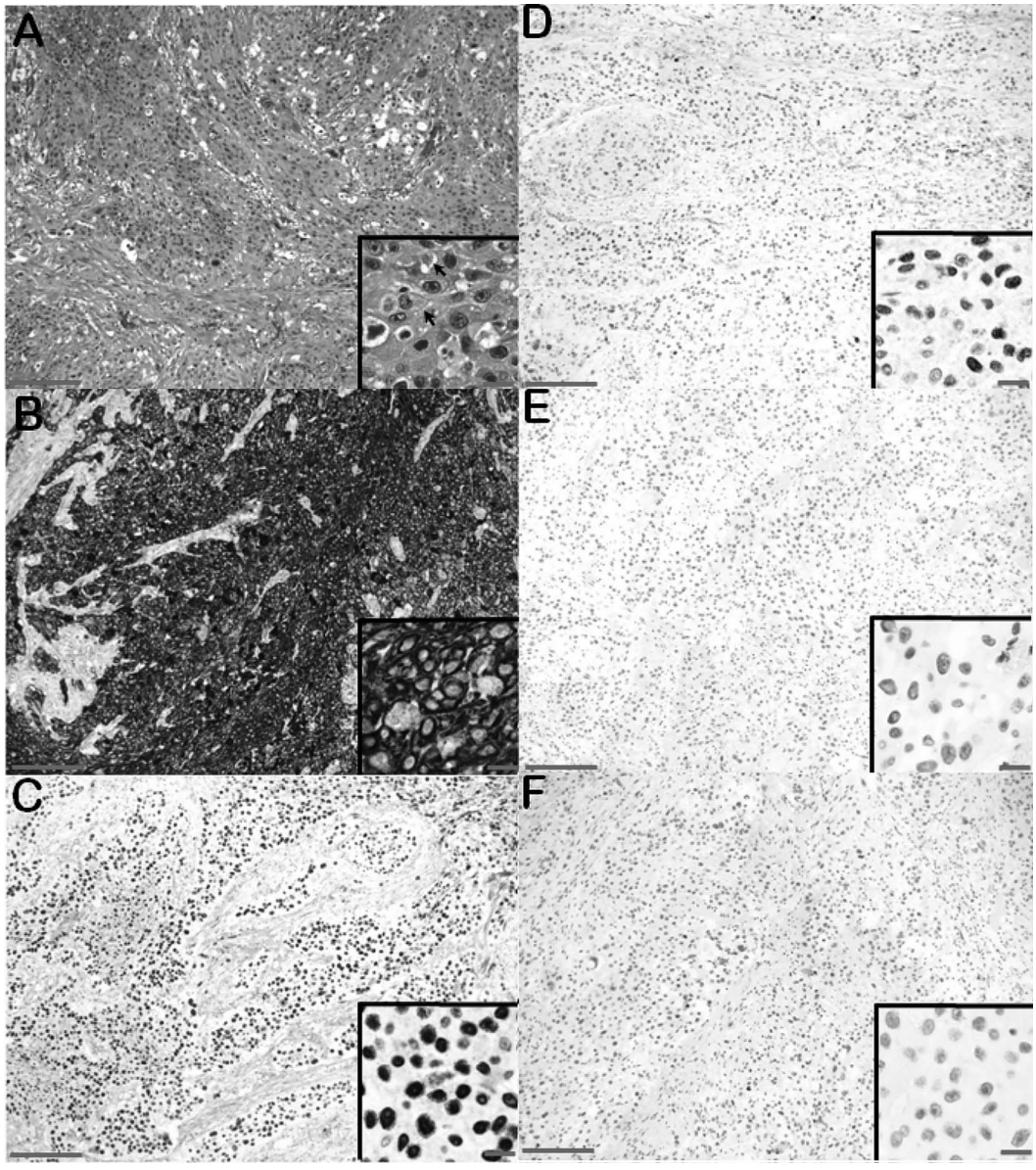

FIGURE 2. North American River otter (*Lontra canadensis*), histology and immunohistochemistry of thoracic masses. (A) The neoplastic cells are arranged in poorly organized packets and lobules supported by a minimal amount of fibrovascular stroma. These cells are round to polygonal, distinctly delineated, with a high cytoplasm to nuclear ratio and a large centrally positioned round-to-oval nucleus containing finely stippled chromatin and a single magenta nucleolus. Inset: Neoplastic cells have distinct intercellular bridges (arrows), H&E stain. (B–F) Neoplastic cells immunohistochemically stained with the use of antisera against high-molecular-weight keratin (HMWK; antibody clone β34E12), p40, p63, calretinin, and TTF-1, respectively. Neoplastic cells show clear cytoplasmic positivity for (B) HMWK and nuclear positivity for both (C) p40 and (D) p63. (E) Calretinin and (F) TTF-1 are both negative. Scale bar, low power image = 400 μm; scale bar, high power image (inset) = 40 μm.

there were carcinomas in situ and invasive multinodular masses (Fig. 1B, C). Tumor emboli were present within lymphatic vessels (Fig. 1D). All of these masses were similar in histologic appearance with absence of a capsule, high cell density, and expansive growth. The neoplastic cells were arranged in poorly organized packets and lobules supported by a minimal amount of fibrovascular stroma. The neoplastic cells were round to polygonal, distinctly delineated, with a large volume of homogenous eosinophilic cytoplasm and a large, centrally positioned round-to-oval nucleus containing finely stippled chromatin and one magenta nucleolus (Fig. 2A). Intercellular bridges were present (presumably desmosomes, Fig. 2A inset, arrows) between neoplastic cells. There was approximately one mitotic figure per every three high-powered fields, and there was moderate to marked anisocytosis and anisokaryosis. There were multiple lytic necrotic foci present with eosinophilic cellular debris and some basophilic nuclear fragments and dystrophic mineralization. The masses were multifocally surrounded by a thin rim of lymphocytes admixed with fewer macrophages. Neoplastic cells showed clear cytoplasmic positivity for HMWK (Fig. 2B and inset) and nuclear positivity for both squamous cell markers, p40 and p63 (Fig. 2C and D with insets, respectively). Calretinin (Fig. 2E and inset) and TTF-1 (Fig. 2F and inset), markers for mesothelioma and pulmonary cells, respectively, were both negative. Pleural papillary projections were composed of reactive mesothelium supported by fibrovascular stroma. Other organs showed no remarkable histologic abnormalities.

The most common origin of thoracic SCC in domestic mammals is pulmonary rather than pleural, and the most frequent neoplasms in both animals and humans arising from the thoracic pleura are mesotheliomas (Caswell and Williams 2016). Although mesotheliomas generally show epithelial, sarcomatous, or biphasic histologic patterns, those features were not seen in the current case. Furthermore, mesotheliomas may arise spontaneously or may be induced by asbestos fibers, but no fibers or other foreign bodies were seen here with a polarizing light filter. For this case, the gross presentation and histomorphologic pleural transition from mesothelium to neoplasia, cellular morphology, and immunohistochemical staining profile, strongly suggested a primary pleural origin. No SCC has been described previously in North American river otters and PPSCC have only been described in a few human case reports (Ronchi et al. 2018).

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