Primary diffuse malignant peritoneal mesothelioma in a striped skunk (*Mephitis mephitis*)

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**ABSTRACT.** A 10-year-old female striped skunk (*Mephitis mephitis*) was admitted with severe abdominal distension and lethargy. Cytological examination of the peritoneal fluid revealed activated mesothelial cells. At necropsy, numerous growing together, projecting, 2 to 20 mm in diameter tawny to white masses were scattered throughout the peritoneum including the mesentery, omentum and intestinal serosa. Microscopically, the tumor was composed of prominent papillo-tubular structures, and immunohistochemically, the spindle to polygonal-shaped tumor cells with nuclear polymorphism were strongly reactive for calretinin. Based on those diagnostic features, the neoplasia was diagnosed as malignant mesothelioma. This is the first case report of mesothelioma in the skunk.

**KEY WORDS:** calretinin, mesothelioma, peritoneum, striped-skunk

Mesothelioma is a rare neoplasm in veterinary medicine, arising from the surface serosal cells of the pleural, abdominal and pericardial cavities and from the tunica vaginalis of the testis; although, human cases are reported more frequently, because of the link between mesothelioma and occupational asbestos exposure. Because the inhalation of asbestos is the most irrefutable cause of mesothelioma, people with greater risk were found in asbestos-related industries: the ship building industry, construction or insulation trades [23]. In accordance with this association, the most common site of mesothelioma in humans is the pleura, accounting for more than 90% of cases [2]. In veterinary cases, peritoneal mesothelioma occurred most often (>50%), followed by pleural (<25%), metastatic (<18%) and pericardial (<10%) mesothelioma [13, 15, 21, 22]. Among pleural mesotheliomas, two cases were found in the peritoneum as well [2, 21]. The association between asbestos and spontaneous mesothelioma in animals is unclear, although there is a case report of a canine mesothelioma, in which high levels of asbestos were detected in the lung tissue of the dog [12]. Most veterinary mesotheliomas are reported in cattle and dogs, but cases have been reported in other various species including cat, pig, horse, rat, goat, blue fox, pigeon and tiger, except for striped skunk [3, 6, 10, 11, 17, 19, 28, 29].

Histologically, veterinary mesotheliomas have three histological types: epithelioid, the sarcomatoid and the biphasic [2, 4, 7, 10, 11, 13, 16, 18, 20]. The epithelioid type is the most common type with large, round to polyhedral cells with abundant cytoplasm and a hyperchromatic and pleomorphic nucleus. The sarcomatoid types primarily consist of elongated fusiform cells, mimicking a sarcoma, and the biphasic types are a mixture [7, 13, 16, 20].

A 10-year-old female striped skunk (*Mephitis mephitis*), which was raised at the Everland Zoological Gardens in South Korea, was admitted to the Kangwon National University Veterinary Teaching Hospital after two months of severe abdominal distension with lethargy, anorexia and inability for physical exercise. Abdominal radiography and ultrasonography confirmed extensive abdominal effusion (Fig. 1a), and serosanguineous fluid was collected through paracentesis. The fluid was pelleted, smeared on slides and stained with May-Grünwald-Giemsa. It contained multiple clusters of activated mesothelial cells with large and multiple nuclei with basophilic cytoplasm and intact erythrocytes and lymphocytes as described elsewhere (Fig. 2) [30]. The blood-tinged ascites, as Parasad et al. described, seemed to be due to bleeding from the tumorous growth [26].

Multiple trials of antibiotic treatments and fluid therapy did not improve the abdominal effusion. The animal was taken to the operating room, and a midline laparotomy was performed. A large amount of serosanguineous fluid was found in the opened abdominal cavity. In more than 90% of the peritoneal mesotheliomas, severe abdominal effusion is a primary clinical sign, which is likely attributed to altered vascular permeability and obstructed lymphatic drainage by the tumor [27]. Numerous projective, multifocal or confluent, tan to white, 2 to 20 mm in diameter firm nodules and plaques thoroughly covered multiple visceral organs, including the omentum, small and large intestines and either pleural or abdominal side of the diaphragm, and abdominal wall (Fig. 1b). The skunk was euthanized during surgery, and a postmortem examination followed.
The abdominal organs affected by the tumors were slightly compressed. Tissue samples from the tumor nodules and representative tissue specimens were collected and fixed in 10% (w/v) neutral buffered formalin and then subjected to a microscopic examination. Immunohistochemical (IHC) analyses were performed as previously described [24], using three primary antibodies: rabbit polyclonal anti-calretinin (H-45; 1:100, Santa Cruz Biotechnology, Inc., Dallas, TX, U.S.A.), mouse monoclonal anti-mesothelin (C-3; 1:50, Santa Cruz Biotechnology, Inc.), mouse monoclonal anti-cytokeratin 5/6 (CK5/6; 1:50, Millipore™, Danvers, MA, U.S.A.) and mouse monoclonal anti-vimentin (RV202; 1:100, abcam®, Cambridge, U.K.). The VECTASTAIN® Universal ABC kit (Vector, Burlingame, CA, U.S.A.) was employed with 3,3′-diaminobenzidine (DAB) chromogen and then counterstained with hematoxylin. Each antibody-treated slide had a corresponding mock-treated slide.

In histological examination, the omentum, mesenteric surface and serosa were markedly covered with multiple layers of cuboidal to polyhedral neoplastic cells with polymorphic nuclei and scarce eosinophilic cytoplasm, forming tubulopapillary outgrowths which were well supported by fibrous connective tissue and diffuse overgrowth inward. The tumor cells had large, pleomorphic and sometimes vesiculated nuclei with predominant nucleoli (Fig. 3a). Very few mitotic figures were viewed. The histomorphological characteristics of the tumor were highly indicative of epithelioid mesothelioma.

Calretinin is an intracellular calcium-binding protein belonging to the Troponin-C superfamilly, which is abundant in the nervous system and also expressed by mesothelial cells and their tumors [8, 31]. Calretinin is regarded as one of the most specific mesothelioma markers. Additionally, CK 5/6 are family members of the intermediate filaments found in all epithelial cells, which are particularly observed in mesothelial cells and are not reactive with adenocarcinoma, making them a reliable differentiating marker for mesothelioma [9, 25]. In this case, the tumor cells were strongly positive for calretinin (Fig. 3b) and positive for CK 5/6, mesothelin and vimentin (data not shown) in both the cytoplasm and nucleus.

For the peritoneal epithelioid mesotheliomas, primary and/or metastatic adenocarcinomas arising from visceral organs should be carefully differentiated [1, 27]. In this case, there was no evidence of any metastatic adenocarcinoma from other organs, and the reactivity with markers for mesothelioma ruled out the possibility of a primary adenocarcinoma.

The cause of the mesothelioma in the stripped skunk remains to be determined. More than 50% of all veterinary mesothelioma cases have been reported in the peritoneum, implying that mesothelioma-causing factors in animals might be different from humans [5, 14]. In this case, asbestos...
fibers were not detected in any of the representative tissue samples, and the epidemiological assessment for exposure to asbestos was negative. To our knowledge, this is the first report of mesothelioma in a striped-skunk in the veterinary medicine.

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