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Case Report / *Olgu Sunumu*

Progressive respiratory distress caused by a laryngeal histiocytic sarcoma in a European rabbit (*Oryctolagus cuniculus*)

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Abstract: Histiocytic sarcoma (HS) represents a rare type of malignant disease, characterized by neoplastic proliferation of interstitial dendritic cells (iDCs). In rabbits, there are only two previous reports of HS with pulmonary and cutaneous primary localizations. A 2-year-old, male, European rabbit (*Oryctolagus cuniculus*) with a clinical history of chronic respiratory distress and progressive weight loss was necropsied. Postmortem examination revealed a gray-white, well-demarcated ovoid mass that deformed the ventral region of the larynx. Similar nodules were identified in both lungs. Histologically, the laryngeal mass and pulmonary nodules were composed of dense sheets of neoplastic histiocytes with marked atypia and multinucleated neoplastic cells. Immunohistochemically, the neoplastic cells were intensely positive for Iba1, CD1a, and vimentin, occasionally for E-cadherin, and negative for multi-cytokeratin. A diagnosis of laryngeal HS with multiple pulmonary metastases was made. To the authors' knowledge, laryngeal histiocytic tumor has not been previously reported in rabbits.

Keywords: Histiocytic sarcoma, larynx, pulmonary metastases, rabbit.

Histiocytic disorders are well-recognized in dogs and cats, and include canine cutaneous histiocytoma, cutaneous and pulmonary Langerhans cell histiocytosis (LCH), cutaneous and systemic reactive histiocytosis, histiocytic sarcoma, feline progressive histiocytosis, and dendritic cell leukemia (9).

Histiocytic sarcoma (HS) represents a rare type of malignant disease, characterized by neoplastic proliferation of interstitial dendritic cells (iDCs), while the hemophagocytic type originates from macrophages (9). In rabbits, there are two previous reports of HS with pulmonary and cutaneous primary localizations (7, 8). A disseminated HS with hemophagocytosis was also previously described in a rabbit (6). These tumors have variable biologic behavior, although the malignant counterparts have a poor prognosis (4).

To our knowledge, there is no previous report on primary laryngeal HS in domestic rabbits. In the present study, we describe the pathological, histological, and immunohistological features of a primary laryngeal HS with multiple pulmonary metastases in a domestic rabbit.

A 2-year-old, male, European rabbit (*Oryctolagus cuniculus*) was referred to the Emergency Department due to chronic respiratory distress and progressive weight loss lasting for two weeks. On physical examination cachexia, tachypnea, sneezing, coughing, and swelling of the ventral side of the larynx were observed. A fine-needle aspiration of the laryngeal mass was performed. Due to the poor prognosis, rapidly deteriorating body condition, and owner's financial limitation, the animal was euthanized and a postmortem examination was performed. Tissue samples from both laryngeal mass and pulmonary nodules were collected for histological and immunohistochemical examination. Tissue samples were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin wax, cut into 3 µm sections, and stained with

hematoxylin and eosin (H&E). For immunohistochemistry, primary antibodies for vimentin (clone SRL33), Iba-1 (polyclonal), CD1a (clone MTB1), E-cadherin (clone 36B5) and multi-cytokeratin (clone AE1/AE3) were used. The samples were automatically processed using Leica Bond-Max system. Negative controls for each sample were prepared by replacing the primary antibody with mouse immunoglobulin G1 negative control. Infiltrating macrophages within the neoplastic mass (for Iba1 and CD1a) and skin, including epidermis and dermis (for multi-cytokeratin, E-cadherin, and vimentin) sampled from the same rabbit served as internal positive controls.

The cytological examination revealed small groups of pleomorphic round cells with distinct borders, abundant pale cytoplasm, high nuclear:cytoplasmic (N:C) ratio and pleomorphic nuclei with 1-2 prominent nucleoli (Figure 1). Binucleated and multinucleated neoplastic cells were occasionally seen. Consequently, a presumptive cytological diagnosis of a round cell tumor was made.

Postmortem examination revealed severe atrophy of the muscle tissue, absence of subcutaneous and retroperitoneal fat, serous atrophy of the adipose tissue at the base of the heart (cachexia), and presence of approximately 20 ml of serous fluid within the peritoneal cavity. A gray-white, well-demarcated, ovoid mass, measuring 1.4x0.9x1.6 cm in diameter deformed and effaced the ventral region of the larynx, including the thyroid and cricoid cartilages, bulged on the ventral laryngeal surface, and partially obliterated the laryngeal lumen. The intraluminal part of the mass showed extensive necrosis, hemorrhages, and ulceration. Moreover, small nodular lesions, ranging from 4 to 10 mm in diameter were randomly distributed in both lungs, mostly affecting the cranial lobes (Figure 2). No similar findings were identified in any other organ.

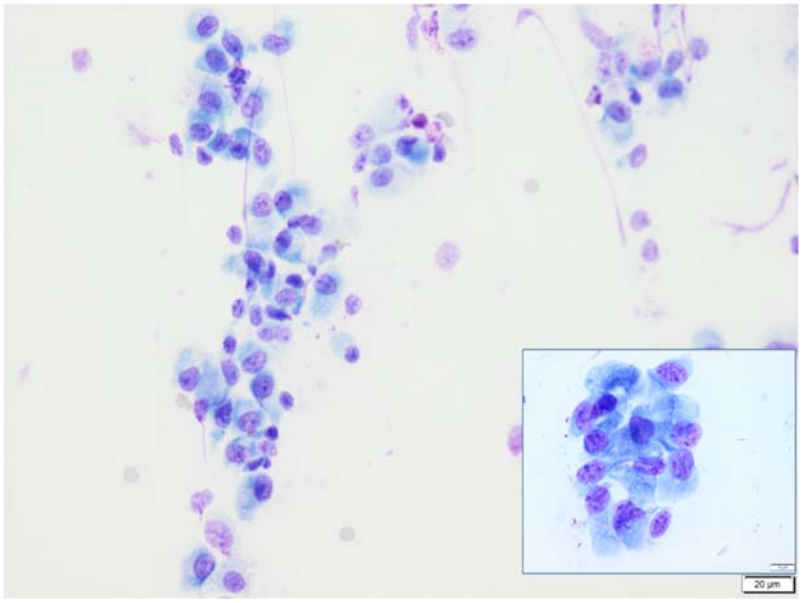

Figure 1. Photomicrograph of a fine-needle aspiration cytology from the laryngeal mass of 2-year-old domestic rabbit. Note many individualized and groups of pleomorphic round cells with abundant pale blue cytoplasm and pleomorphic nuclei. The inset shows atypical cells characterized by marked anisokaryosis and multinucleated cells (central area). Diff-Quick stain. Bar=20 µm.

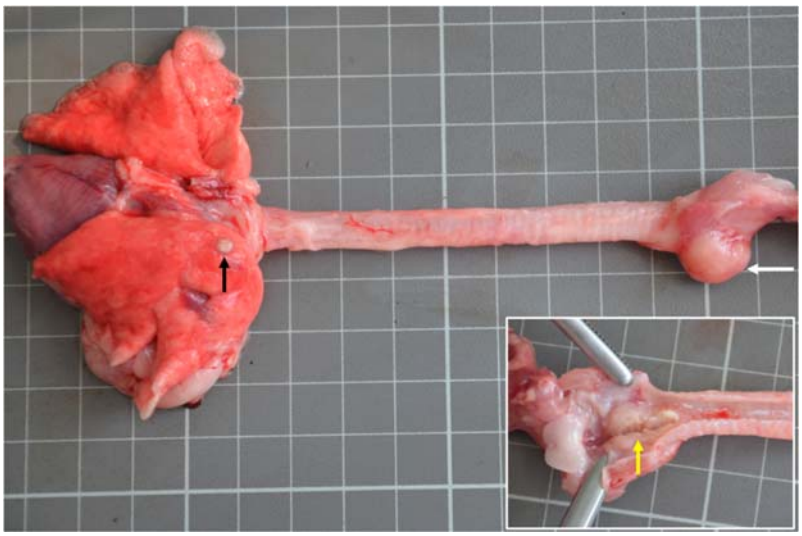

Figure 2. Gross features of the laryngeal histiocytic sarcoma and pulmonary metastases in a 2-year-old domestic rabbit. The laryngeal lumen is partially obliterated by an ulcerated neoplastic mass, infiltrating the laryngeal wall (the inset, yellow arrow) and bulging on the laryngeal serosa (white arrow). The pulmonary parenchyma is affected by randomly distributed and variably sized nodules (black arrow).

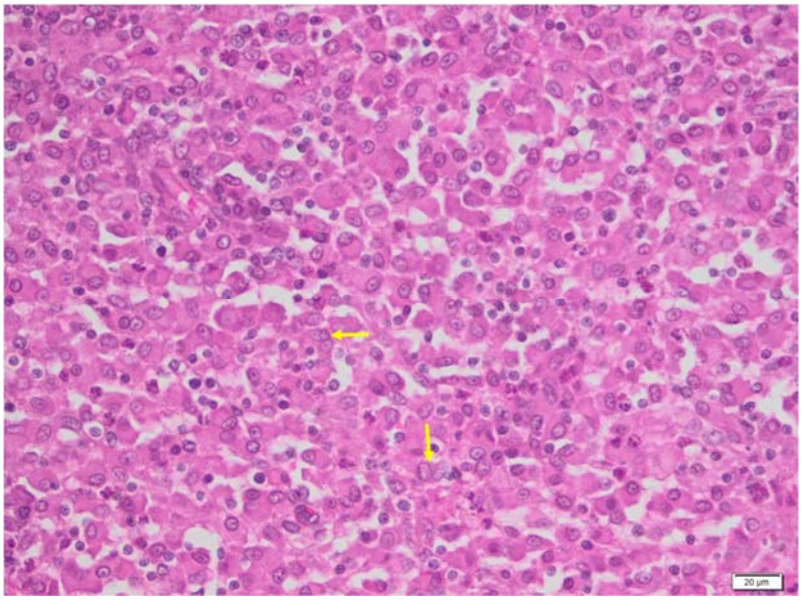

Figure 3. The laryngeal mass is composed of dense sheets of round to polyhedral cells with variably distinct borders, abundant eosinophilic cytoplasm, and oval to irregular nuclei; scattered binucleated neoplastic cells (yellow arrows) are also seen. H&E stain. Bar=20 µm.

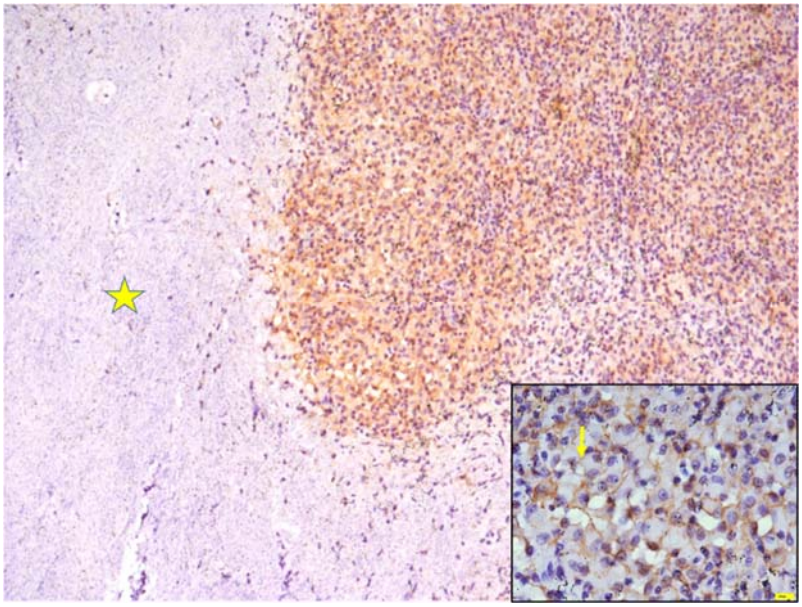

Figure 4. The neoplastic mass is diffusely immunopositive for Iba1, while the necrotic areas are negative (yellow star). Neoplastic cells showing diffuse and intense membranous immunoreactivity (the inset, yellow arrow). IHC. Bar=10 µm.

Histologically, the laryngeal mass extended from the mucosa to the serosa, infiltrating and effacing the ventral laryngeal cartilages and muscle tissue. The unencapsulated tumor was densely cellular, composed of pleomorphic cells arranged in sheets, and separated by a scant fibrovascular stroma (Figure 3). The cells were polygonal to round with variably distinct borders, moderate amounts of pale eosinophilic cytoplasm and high N:C ratio. The nuclei were pleomorphic, round, oval or indented with finely granular chromatin and 1-2 prominent nucleoli. Binucleated and multinucleated neoplastic cells were also observed. Mitotic figures varied from 1 to 5/high power field. Additionally, small areas of necrosis, hemorrhages, and mild infiltration of lymphocytes and heterophils were noticed. The pulmonary nodules showed similar histological features.

Immunohistochemically, the neoplastic cells showed an intense and diffuse reaction for Iba-1 (Figure 4), CD1a, and vimentin, and occasionally for E-cadherin. The neoplastic cells were negative for multi-cytokeratin. Based on the cytological, histological, and immunohistochemical results, the tumor was diagnosed as histiocytic sarcoma with multiple pulmonary metastases. The immunohistochemical results of pulmonary metastases were similar to those of the primary laryngeal tumor.

Respiratory disorders are the second most common health disorder in domestic rabbits after gastrointestinal diseases. Dyspnea is a very common clinical finding in rabbits and is frequently associated with various infections of the respiratory tract. Pasteurellosis is the most important respiratory disease affecting domestic rabbits,

but other bacteria, including *Bordetella bronchiseptica* and *Staphylococcus* spp. are significant opportunistic pathogens (3). In our case, physical examination and cytological evaluation of the laryngeal mass excluded a subcutaneous abscess caused by a bacterial infection.

Spontaneous neoplasia in lagomorphs are rare, accounting between 0.5% and 2.7% (13). Similarly to humans and other species, the incidence of tumors in rabbits increases with age, being up to 8.4% after 2 years of age (13).

Primary laryngeal tumors are rarely encountered among all animal species. In canine and feline patients, laryngeal tumors account only for 0.02% in dogs and 0.14% in cats (14). In dogs, laryngeal tumors are mostly represented by squamous cell carcinoma (14), rhabdomyosarcoma (11) and chondroma (10), while in cats squamous cell carcinoma and lymphoma (12) are the most commonly encountered laryngeal tumors.

In rabbits, laryngeal and tracheal tumors are considered uncommon causes of progressive dyspnea. A recent report described two cases of airway obstruction caused by laryngeal osteochondroma and tracheal adenocarcinoma (2). To the authors' knowledge, laryngeal histiocytic neoplastic diseases have not been previously reported in rabbits.

Histiocytic disorders, as neoplastic or inflammatory processes, are well documented in humans, dogs, and cats. In dogs, HS complex includes two forms, localized and disseminated HS, characterized by infiltration of neoplastic histiocytes with marked cytological atypia, multinucleated cells, and high mitotic index (9). Localized HS affects the skin and subcutis, periarticular tissues, spleen, lungs, lymph nodes, and bone marrow (9). The present case was diagnosed as localized laryngeal HS, with multiple pulmonary metastases, showing similar histological and immunohistochemical features as those described in other cases of pulmonary (8) and cutaneous (7) HS in rabbits. Regarding the behavior of localized HS, our findings are in agreement with previous studies (1, 7), demonstrating local tissue invasion and metastases.

Cells of origin for HS are iDCs, which usually express some specific leukocyte surface molecules, including CD1a, MHC class II, and CD11c/CD18 in dogs (1) and Iba1, vimentin, E-cadherin, CD204, and Ki67 in rabbits (7, 8). Immunohistochemistry is extensively used for identification of the origin of HS, but the immunohistochemical differentiation between the localized and disseminated forms of the disease is still debatable (1). In rabbits, due to the fact that there are only a limited number of reported cases of HS, a complete immunophenotype characterization is not yet established. In our case, the definitive diagnosis of HS was made based on morphological features and the expression of vimentin, Iba1, of vimentin, Iba1, and CD1a.

Despite unclear etiopathogenesis of the HS in animals, some previous studies demonstrated various deletions of the tumor suppressor genes (CDKN2A/B, RB1, and PTEN) in certain dog breeds (5). No studies concerning the etiology of HS in domestic rabbits are available.

To conclude, HS is a rare and very aggressive tumor that can affect domestic rabbits, characterized by progressive respiratory distress and poor prognosis if localized in larynx. Therefore, laryngeal HS should be included in the list of differential diagnoses for progressive respiratory disorders in domestic rabbits. Predisposing factors for HS have not been identified in domestic rabbits.

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Ethical Statement

The current study is not an experimental part on living animals, therefore any approval from the ethic committee was not required.

Conflict of Interest

The authors declared that there is no conflict of interest.

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