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Extra-nodal follicular lymphoma in the lung of a free-ranging red deer (*Cervus elaphus*)

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Running head: Lymphoma in a deer

Abstract. A hunted free-range female red deer (*Cervus elaphus*) from a region near the Nahuel Huapi National Park, Northern Patagonia, Argentina, had a focally extensive peribronchial lymphoid proliferative lesion in the lung characterized by multiple follicles lacking mantle zone cells. On examination of immunohistochemically stained tissues a predominance of B-cells (CD 20 positive) with only a few scattered T-cells (CD3 positive) were present. The histologic and immunohistochemical characteristics are consistent with follicular lymphoma, which is frequently seen in humans and less frequently in domestic animals. This appears to be the first report of follicular lymphoma in a deer.

Key words: CD3, CD20, follicular lymphoma, inmunohistochemistry, red deer

Follicular lymphomas are slowly progressive tumors that are recognized by characteristic follicular architecture and fading mantle cell cuffs.[4] These neoplasms have been described in humans, and amongst animals, most frequently in cats and cattle and less commonly in dogs.[4] While in humans this type of neoplasm represents up to 25% of all lymphomas diagnosed, it is thought that less than 1% of lymphomas diagnosed in animals are of the follicular type.[4] However, to the best of our knowledge, this condition has not been previously reported in deer. Further, follicular lymphomas have not been reported in the lung of any animal species. We present here the diagnosis of what appears to be a primary lung follicular lymphoma in a free ranging red deer.

An approximately 2 year-old, 100 kg female red deer was shot by a hunter in the Nahuel Huapi National Park, Northern Patagonia, Argentina. No obvious clinical or external gross abnormalities were reported by the hunter. The body was transported to a meat smoking facility where veterinary inspection was performed. During this process, one of the authors of this report (Chang Reissig) had access to the carcass and abdominal and thoracic viscera, which were examined as part of a health surveillance project of wild ungulates performed in the Nahuel Huapi National Park. No significant gross abnormalities were observed during the gross examination of the visceral organs, lymph nodes or the carcass. Samples of liver, heart, spleen, kidneys, lymph nodes (prescapular, popliteal, bronchial, mediastinal, submandibular, retropharyngeal and mesenteric), fore-stomachs, abomasum, small and large intestine, and both lungs (all lobes) were collected and fixed by immersion in 10% buffered formalin, pH 7.2, for several weeks before being embedded in paraffin wax, sectioned at 4 µm, and stained with hematoxylin and eosin. Immunohistochemistry for CD3, CD18, CD20, CD79 and pancytokeratin was performed using an Avidin biotin conjugate (ABC) immunostaining method.[a]

Histologically, a single mass located eccentrically around a bronchiole was observed in the lung (Fig. 1). At the sub-microscopic level, this mass was ~ 0.5 mm diameter, roughly circular, well circumscribed, encapsulated and was compressing the adjacent parenchyma and the lumen of an adjacent bronchiole. Histologically, the mass was composed of a more or less uniform population of predominantly small to medium size cleaved lymphocytes with densely stained nuclei and usually no nucleoli, and scant, pale cytoplasm (Fig. 2) that resembled the centrocytes of low grade follicular lymphoma as seen in dogs.[4] The nuclei of these cells were ~ 8-10 µm in diameter. The small lymphocytes were mixed with a much smaller number of large round cells of open nuclei with peripheralized chromatin and 1 to 3 nucleoli (Fig. 2). These cells were interpreted to be centroblasts, which are considered to be the main cell type in high grade follicular lymphoma.[4] The centroblasts were ~14-18 µm in diameter. There was mild to moderate anisocytosis and anisokaryosis and the mitotic rate was less than 1 per HPF (Fig. 2). The follicular cells were closely aggregated, with the smaller cells being uniform in size, but of irregular shape. The follicles were closely facetted and separated from each other by thin fibrous trabeculae that contained blood vessels. Identical cell types were present in every follicle. Most of the follicular cells showed strong positive cytoplasmic staining for CD20 (Fig. 3) and only a few cells showed weak to moderate cytoplasmic staining for CD79 (Fig. 4). Randomly throughout the neoplasm there were a few round cells that showed moderate cytoplasmic positive staining for CD3 (Fig. 5), while CD18 and pancytokeratin (Fig. 6) were negative. No mantle cells were observed around the follicles, and no necrosis, apoptosis, inflammatory infiltrates, tingible body macrophages or hemorrhage was seen. The parenchyma surrounding the mass was atelectatic, but apart from this, no other histological abnormalities were observed in any of the sections of lung examined.

No metastasis or other neoplastic changes were observed in other organs examined. Few, ~ 100 µm x 50 µm protozoal cysts with morphology consistent with *Sarcocystis* spp.

were present within cardiomyocytes in the heart, but these parasites were not associated with significant tissue damage or inflammation.

Based on the histological morphology of packets of lymphoid cells compressing the pulmonary parenchyma and the adjacent bronchiole, plus the monomorphic nature of the cell population, the absence of mantle cell cuffs and apoptotic cells, and the positivity of the cells to CD20, this neoplasm was diagnosed as follicular lymphoma.[4] The cell morphology described in this case is compatible with cases of human follicular lymphoma where the cells inactivate the apoptotic gene, with the consequence that there are no dying cells and hence no macrophages are seen, such as one would expect to observe in benign follicles with progressive B-cell selection and frequent cell death.[4] By definition, follicular lymphomas are neoplams of follicular center B-cells (centrocytes and centroblasts) that have a follicular pattern and in which the centrocytes fail to undergo apoptosis, due to chromosomal rearrangement.[4]

Multiple samples from all lobes of both lungs, lymph nodes and most visceral organs of this animal were collected and examined, and no lesions were observed apart from the neoplasia described here. It is therefore likely that this neoplasia was unicentric. Strong positivity for CD20 indicated that this was a B-cell lymphoma. Only a few cells were positive for CD79. These results indicate that in deer CD20 seems to be a better marker for B cells than CD79, or alternatively, that CD79 is not strongly expressed in lymphocytes of this species.

Follicular lymphomas are predominately of lymph node origin.[2, 4] However, extranodal lymphoid tissue including spleen, oropharynx, bone marrow, liver and less commonly non-lymphoid organs have been involved.[2, 4] We are not aware, however, of any follicular lymphoma previously diagnosed in the lung of any animal species. This case is

particularly unique because of the presence of the largest tumor formation around a bronchiole, which suggests that the origin of the neoplasia was in the cells of the BALT.

The main differential diagnosis for follicular lymphoma is benign follicular hyperplasia.[1] In this case, follicular hyperplasia was ruled out because the follicles were more or less uniform in size (follicular hyperplasia usually has more variation in size) and there was no mantle cell cuff around each germinal center. In addition, there was no antigen related polarity present that would indicate a benign process, and all follicles had the same cellular composition.[1,3] Also, as noted above, the moderate mitotic activity, which is characteristic of follicular hyperplasia, was absent. Finally, there was absence of the starry-sky pattern of tingible body macrophages, normally seen within benign germinal centers.[3]

In this case, no clinical alterations were reported by the hunters in the brief period during which this animal was observed before it was shot. Although this cannot be considered a clinical examination, the fact that this animal was in very good nutritional condition suggests that no major clinical alterations occurred, as loss of condition tends to be one of the first indications of chronic health problems in wild animals.[5] Follicular lymphomas are indolent lesions that may reach quite large size and it is thought that in animals they might become more aggressive with time, an outcome usually seen in humans.[1,4] It is possible that this might have been the case with this deer had it not been killed at this stage of the neoplasm development.

Sources and manufacturers

a. Dako, Carpinteria, California, US

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figure legends

Figure 1. Red deer. Follicular lymphoma located eccentrically around a bronchiole and compressing the surrounding pulmonary parenchyma. Observe the absence of mantle cells cuff and of tingible body macrophages. HE. Bar = 100 µm.

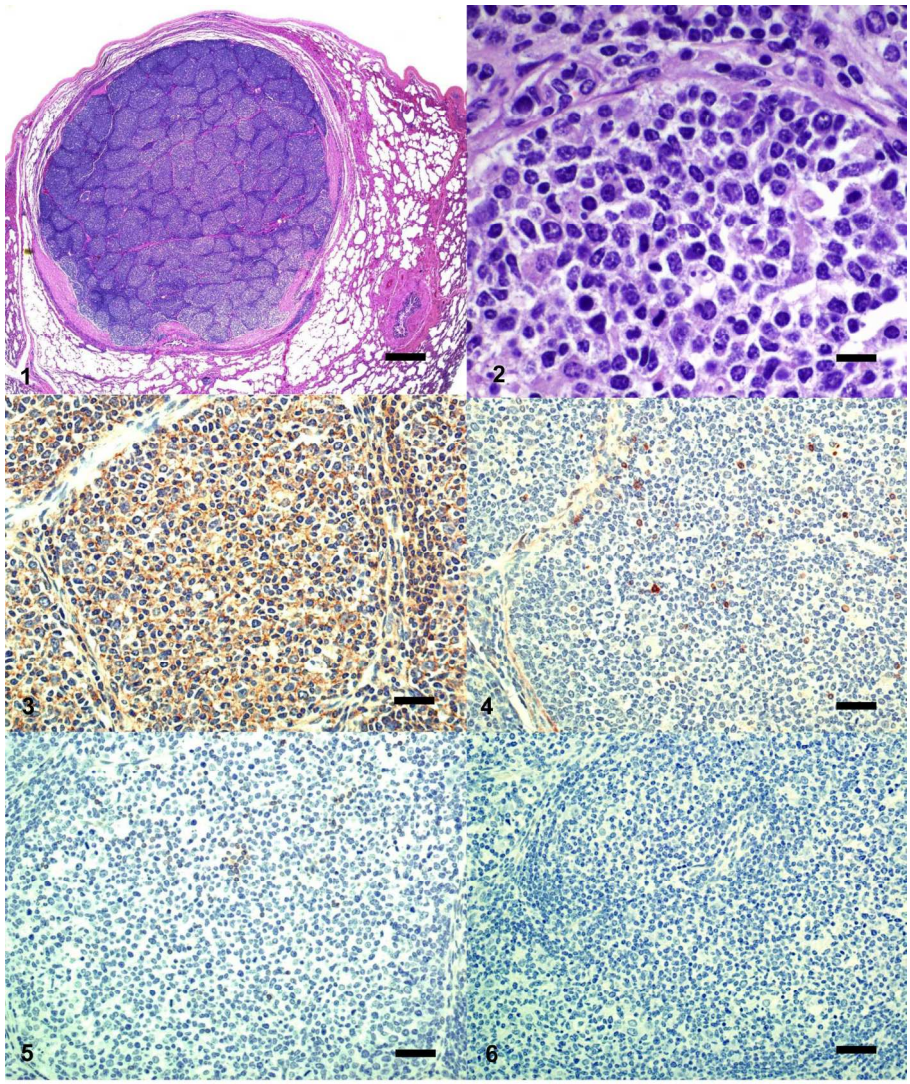
Figure 2. Red deer. High magnification of the follicular lymphoma showed in Figure 1. Observe monomorphic population of mostly small lymphocytes and the absence of tingible body macrophages. HE. Bar = 20 µm.

Figure 3. Red deer. CD20 staining. Most cells are positively stained. Bar = 50 µm.

Figure 4. Red deer. CD79 staining. Only a reduced number of cell cells is positively stained. Bar = 50 µm.

Figure 5. Red deer. CD3 staining. Only a reduced cell of cells is positively stained. Bar = 50 µm.

Figure 6. Red deer. Pancytokeratin staining. No staining is observed. Bar = 50 µm.



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