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## Malignant Lymphoma in a West Indian Manatee (*Trichechus manatus*)

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**ABSTRACT:** We identified a malignant lymphoma infiltrating the lung, liver, kidney, mesenteric lymph nodes, and eye as the cause of death in a male West Indian manatee (*Trichechus manatus*). Diagnosis was based on gross, histopathologic, and immunohistochemical studies. Tissue samples from ten organs were included in a tissue microarray and sections from this array were subjected to immunohistochemical staining. The cytoplasm of the neoplastic lymphocytes identified in six organs was positive for CD3, a marker for T-cell differentiation. The neoplastic cells were negative for CD79α, a marker for B-cell differentiation. The cause of this neoplasm was not determined. This is the first report of malignant lymphoma in the mammal order Sirenia.

**Key words:** Case history, lymphoma, tissue microarray, *Trichechus manatus*, West Indian manatee.

The West Indian manatee (*Trichechus manatus*) is rare in both zoologic collections and in the wild. Consequently, few pathologic descriptions of disease in this animal are available that include both diagnostic and medical data. Death due to infectious diseases seems to be a relatively rare occurrence in free-ranging manatees. In manatee necropsies where a cause of death could be ascertained, the cause was most frequently associated with environmental factors such as human-inflicted trauma, cold winters, and watercraft casualties. Large numbers of manatee deaths remain undetermined (Powell et al., 1981; Buergelt et al., 1984; O'Shea, 1988; Montoya-Ospina et al., 2001; Bossart et al., 2002). There are no previous reports of lymphoid neoplasia in the mammal order Sirenia, although lymphoid neoplasms have been described in a wide range of other mammals. This report describes a

case of T-cell lymphoma in a West Indian manatee.

A male West Indian manatee was imported from Georgetown Zoo, Guyana, to Odense Zoo, Denmark, in 2002. The animal measured 217 cm from nose to tail and was approximately 5 yr old on arrival. During the subsequent two years, the manatee developed cutaneous papillomas on the back covering an area about 5 cm in diameter. The manatee seemed more affected by this skin condition than the other manatees in the same exhibition. In 2004, at 7 yr of age, the manatee exhibited clinical signs of anorexia, weight loss, and mild respiratory difficulty. The animal was isolated, and repeated hematologic and biochemical testing was performed. Examination of the peripheral blood showed leukocytosis. Clinicopathologic investigation, including bacterial culture and parasitologic analyses of fecal and blood samples, found no specific cause of disease. The symptoms did not respond to either repeated broad-spectrum antibiotics or to supportive treatment with glucocorticoids, vitamins, and fluid. The manatee was found dead after 5 wk of treatment.

At necropsy, the manatee weighed 185 kg. The skin had several randomly distributed, small circular areas of superficial hyperkeratosis (sequelae to papillomatosis). The dermis on incision was approximately 8–10 mm thick. The animal was emaciated, the thickness of subcutaneous adipose tissue generally measuring 5–15 mm or less. Abdominal fat deposits were reduced, although moderate amounts of fat were visible around the kidneys. The muscles were white to pale red. Both focal and co-

alescent areas of necrosis and hemorrhage were seen in the musculature (apparently a local reaction to repeated intramuscular injections), affecting a broad area approximately 40 cm wide across the caudal part of the back. Thoracic lymph nodes appeared enlarged. However, taking into account the general size of lymph nodes in marine mammals this enlargement was probably mild. The lungs were congested and the caudal part of the lobes had extensive emphysema. The kidneys showed numerous irregular pale spots up to 1 cm in size. The liver capsule appeared thickened and it included irregular fleshy nodules up to 0.2 cm to 3 cm in size. The liver parenchyma appeared normal. All other organs were grossly unremarkable.

Tissue samples from skin, muscle, lung, heart, liver, spleen, stomach, intestine, kidney, thoracic, and abdominal lymph nodes, and the eyes were collected in phosphate-buffered 4% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and Ziehl-Neelsen for histologic examination. Tissue cores (2 mm in diameter) were removed from each paraffin-embedded tissue block using a Tissue Microarrayer (Beecher Instruments) and collected in a new paraffin tissue microarray (TMA) block, essentially as described (Kononen et al., 1998). The TMA consisted of four cores from 10 organs (skin, skeletal muscle, lung, kidney, adrenal gland, liver, spleen, mesenteric lymph node, small intestine, and pancreas). Ten cores sampled from normal manatee lymph node tissue were included as positive controls.

Four 4- $\mu$ m sections were cut from the TMA and deparaffinized. Antigen retrieval was performed by heating the sections in a microwave, at maximum power for 15 min in high pH buffer (Tris-EGTA, pH 9). Sections were stained by immunohistochemistry using commercially available antibodies (DakoCytomation, Glostrup, Denmark) to B cells (CD79 $\alpha$ ; HM57) and T cells (CD3; F7.2.38). The optimal dilutions used for the primary antibodies (1:40

for CD79 $\alpha$ , 1:60 for CD3) were determined by titration using whole sections of normal lymphoid tissue from a manatee. Normal mouse serum was substituted for the primary antibodies as a negative control. The EnVision system (DakoCytomation) was used for visualization. The WHO histologic classification of hematopoietic tumors of domestic animals (Valli et al., 2002) was used to define the lymphoma.

Microscopy showed neoplastic lymphoid cells infiltrating multiple organs. These cells had medium to large nuclei, with a coarse chromatin structure, several small nucleoli, and scant cytoplasm. Mitotic figures were frequent (two to four per high-power field). The infiltrate was associated with delicate sclerosis and admixed with scattered histiocytes and plasma cells. Very few eosinophilic granulocytes were seen, probably representing pseudoeosinophils, the primary granulocyte of manatees (Bazzini et al., 1986). Granulomas were not seen in any of the organs and Ziehl-Neelsen-stained tissue sections were negative for acid-fast mycobacteria.

The lungs contained multiple neoplastic nodules made up primarily of atypical lymphoid cells, admixed with scattered plasma cells and histiocytes. These nodules were well circumscribed and nonencapsulated, but invaded focally into the surrounding lung tissue. In the mediastinal lymph nodes and spleen, the normal architecture was diffusely obliterated by an infiltrate of atypical lymphoid cells. Multifocal infiltrates of neoplastic lymphoid cells were present in the perinodal connective tissues, in the renal parenchyma, and in periportal areas of the liver. The eye was infiltrated with similar atypical lymphoid cells, with scattered plasma cells and histiocytes. While deeper parts of the corneal stroma were unremarkable, the neoplastic lymphoid cells had infiltrated the anterior part of the corneal stroma, the superficial perilimbal connective tissues, the iris and corpus ciliare, and the surrounding sclera with extension into the chorioidea.

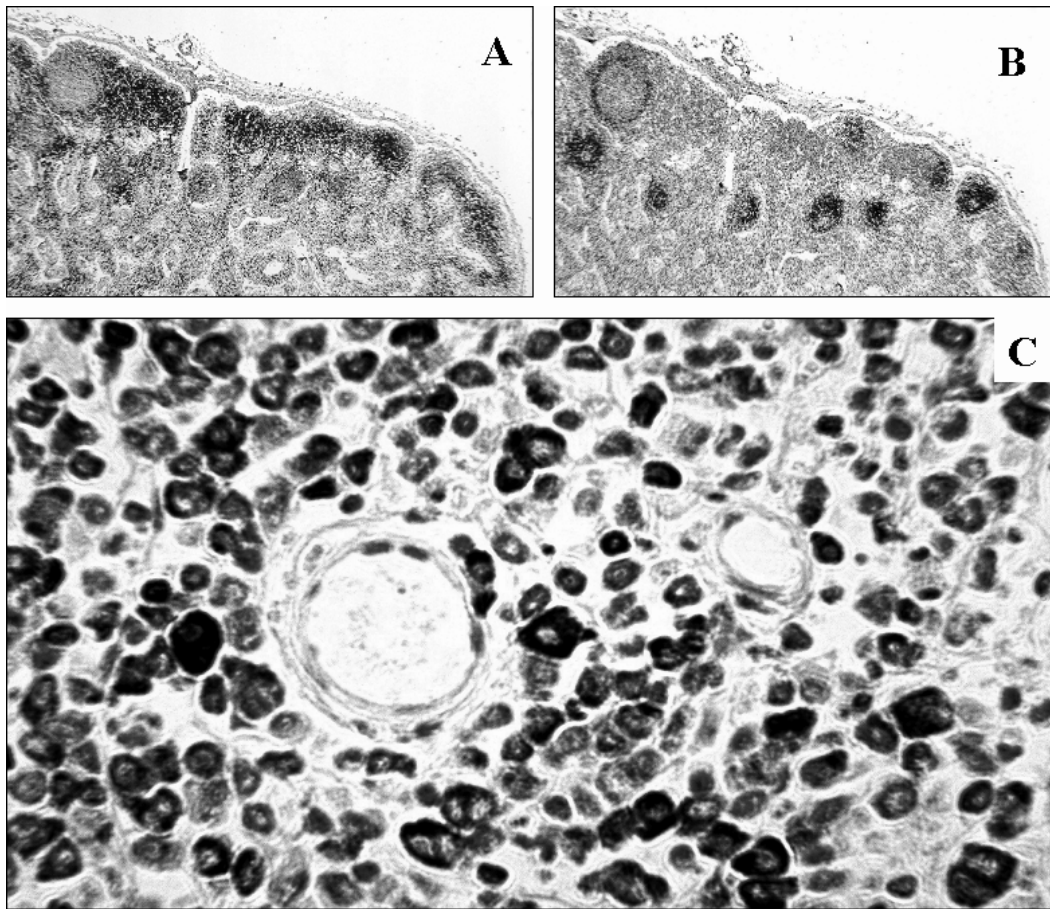


FIGURE 1. A: Immunohistochemical stain of same lymph node showing lymphoid cells positive for a marker for T-cell differentiation: CD3/F7.2.38 (Dako), dilution 1:100, visualization by EnVision (Dako). 20 $\times$ . B: Immunohistochemical stain of normal lymph node of West Indian manatee showing lymphoid cells positive for a marker for B-cell differentiation: CD79 $\alpha$ /HM57 (Dako, Glostrup, Denmark), dilution 1:100, visualization by EnVision (Dako). 20 $\times$ . C: Immunohistochemical stain of small neoplastic process in the lung of West Indian manatee diagnosed with malignant lymphoma. The cytoplasm of near to 100% of the cell infiltrate was positive for a marker for T-cell differentiation: CD3 (Dako), dilution 1:100, visualization by EnVision (Dako). 200 $\times$ .

The reactivity of antibodies directed against human CD3 and CD79 $\alpha$  antigens in manatee lymphocytes was evaluated in normal lymphoid tissue. Immunohistochemical staining of whole sections of formalin-fixed normal manatee lymphoid tissue showed specific staining of T lymphocytes (Fig. 1A) and B lymphocytes (Fig. 1B) with CD3 and CD79 $\alpha$ , respectively.

Immunohistochemical staining of TMA sections revealed neoplastic lymphocytic infiltrates in 6 of 10 tissues represented (lung, spleen, mediastinal lymph nodes,

liver, kidney, eye). The cytoplasm of essentially all identifiable neoplastic cells was labeled positively for CD3 (Fig. 1c). A minor background population of scattered reactive B cells stained positive for CD79 $\alpha$ . A diagnosis of multicentric peripheral T-cell non-Hodgkin lymphoma was made.

It remains unknown if this case represents a rare condition in this species. Although we have encountered anecdotal descriptions of other cases of lymphoid neoplasms in manatees, these have not

been published and could not be otherwise confirmed. Although the tumor in this case was clearly widespread and aggressive, it was not readily detectable clinically, and the correct diagnosis could not be made on gross pathologic examination alone. Thus, cases such as this would be undiagnosed in surveys in which necropsy did not include thorough histopathologic evaluation of tissue samples. Immunohistochemistry proved central in confirming the diagnosis of lymphoma, and in determining the T-cell phenotype. By using TMAs, immunohistochemical evaluation of a large number of tissue specimens could be performed in parallel, while reducing the overall cost and time taken for the analysis. This technique may be useful in future studies of tumors in this and other species, especially when high-throughput studies involving large numbers of tissue samples are required. Relatively small TMAs, such as the one built for this study, can be constructed using commercially available handheld microarrayers, which are significantly less expensive than the equipment used in this study.

The manatee showed antemortem evidence of leukocytosis. These specimens were not kept, and since neither blood nor bone marrow from the necropsy was available for evaluation, we cannot determine whether there was evidence of spread of the lymphoma to the peripheral blood or bone marrow.

The etiology of the lymphoma in this manatee is unclear. In domestic species, the cause of most lymphomas is also unknown. However, a minority are associated with viral oncogenesis (Cullen et al., 2002). T-cell lymphomas have been described in several species, primarily in association with herpesviruses, for example, the Marek's disease virus in poultry (Churchill and Biggs, 1967; Nazerian et al., 1968), and retroviruses, for example, the feline leukemia virus of cats (Hoover and Mullins, 1991; Rezanka et al., 1992), the simian and the human T-cell leukemia virus type 1 (Slattery et al., 1999). A number

of these T-cell lymphomas are associated with either pre-existing, or virus-induced immunodeficiency. The manatee we describe was diagnosed with papillomatosis before death. Previous studies in Florida manatees suggest that such papillomas are caused by a virus closely related to human papilloma virus and that the pathogenesis of the disease may involve activation of latent virus infection in the setting of immune suppression (Bossart et al., 2002). There is no evidence that papilloma viruses may be directly linked to the development of malignant lymphoma. However, the presence of severe papillomatosis may be a marker of underlying immunosuppression in this manatee, thus providing some circumstantial evidence that an unidentified oncogenic virus may have played a role in the development of T-cell lymphoma in this animal.

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