A CASE OF EQUINE MULTICENTRIC LYMPHOMA: CLINICAL, MICROSCOPICAL AND MOLECULAR FINDINGS

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INTRODUCTION

Lymphoma represents a heterogeneous group of haematopoietic tumours originating in lymphoid tissue. Although uncommon, multicentric lymphoma remains the most prevalent form in horses. Recently, Equine Herpes Virus-5 (EHV-5) infection has been associated with lymphoproliferative diseases in young horses[1]. The pathogenesis of equine lymphoma is still poorly understood. There is no predilection for breed or sex and any age can be affected. However, a greater predisposition for horses with 5-10 years of age has been identified. In this species, clinical signs common to all forms of lymphoma include weight loss, fever, lethargy, swelling of the ventral body wall or distal limbs and lymphadenopathy. The diagnosis and staging involves physical examination, abdominal ultrasound, thoracic radiographs and cytology of lesions but the definitive diagnosis is made by histopathologic examination of the biopsies, that is the gold standard method.

The prognosis of lymphoma in horses is poor. Nevertheless, in order to increase the survival time, surgical excision, radiation and chemotherapy are therapeutic possibilities. This study investigated the clinical, pathological and molecular features of a case of equine multicentric lymphoma[2].

CASE DESCRIPTION

- A 5-year-old crossbreed mare was admitted in ICBAS-Equine Clinical Center presenting the following clinical signs :

Lymphadenomegalia	Supraorbital edema	Fever and tachypnea

-Blood laboratory tests were done and revealed:

lymphocytic leukocytosis	Thrombocytopenia	↓ Decreased albumin (2,47 g/dL)	↑ High total protein (9,5 g/dL)

MATERIALS AND METHODS

NECROPSY
Lung, lymph node, heart, diaphragm, gastrointestinal tract and abdominal muscle were colected.

HISTOPATHOLOGY
H&E
Grocott's methamine silver and Periodic acid Schiff (PAS) satinings were performed.

EHV-5 PCR analysis and sequencing was performed in lymph node and pulmonary neoplastic lesions.

Immunohistochemistry panel

Antibody	Clone	Supplier	Dilution	Antigenic Recovery	Incubation
CD3	Polyclonal 1A4	DAKO	1:50	RS/ WB for 30 min.	ON
KI67	Monoclonal MIB-1	DAKO	1:50	RS/ WB for 30 min.	ON
CD79-α	HM-57	Leica Biosystems	1:50	RS/ WB for 30 min.	ON
CD20	L26	DAKO	1:50	RS/ WB for 30 min	ON
PAX-5	1EW	Leica Biosystems	1:40	RS/ WB for 30 min	ON
PD-L1	ab233482	Abcam	1:150	RS/ WB for 30 min	ON
C-KIT	CD117	Leica Biosystems	1:450	RS/ WB for 30 min.	ON

Legend: ON: Overnight, RS/WB: retrieval solution/ water bath

RESULTS

Necropsy Evaluation

Figure 1: **a)** Lymphadenopathy of the mandibular lymph nodes; **b and c)** Multiple and well circumscribed white to brownish nodular lesions located in oropharynx and trachea; **d, e and f)** Lesions and petechiae distributed along the serous surface of diaphragm, pericardium and heart; **g)** petechiae and edema in the gastrointestinal tract and mesentery and enlarged mesenteric lymph nodes.

Microscopic Evaluation

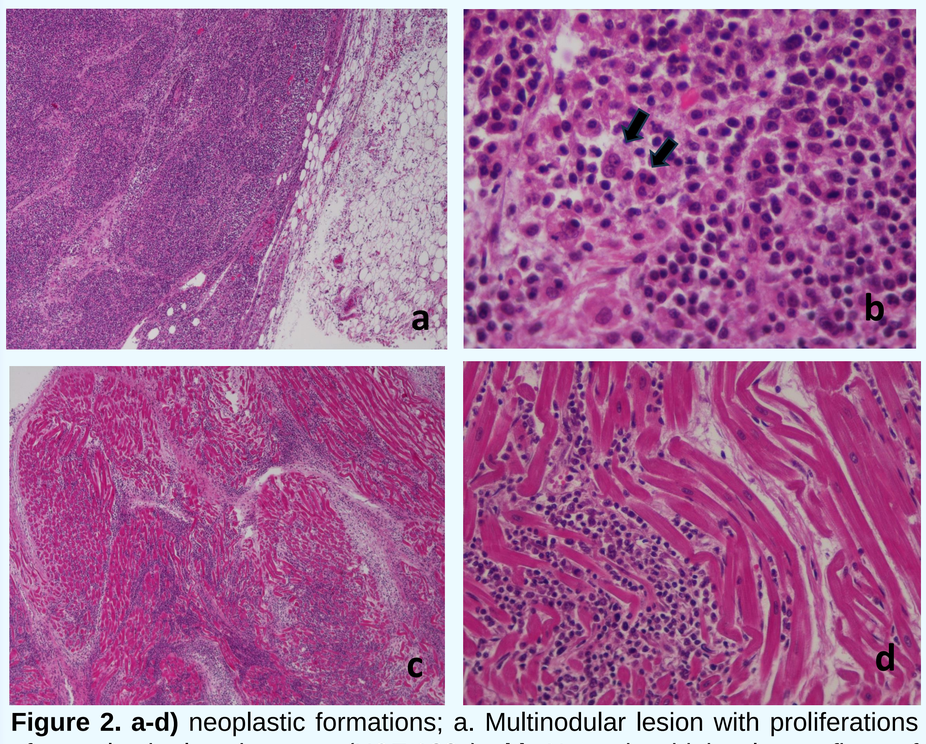

Figure 2. a-d) neoplastic formations; a. Multinodular lesion with proliferations of neoplastic lymphocytes (H&E,100x); **b)** Note the high pleomorfism of neoplastic cells and numerous multinucleated cells (arrows) (H&E, 600X); **c) and d)** Diffuse infiltration of cardiac muscle tissue by neoplastic cells (H&E,40X); d. (H&E,600X).

Immunohistochemistry

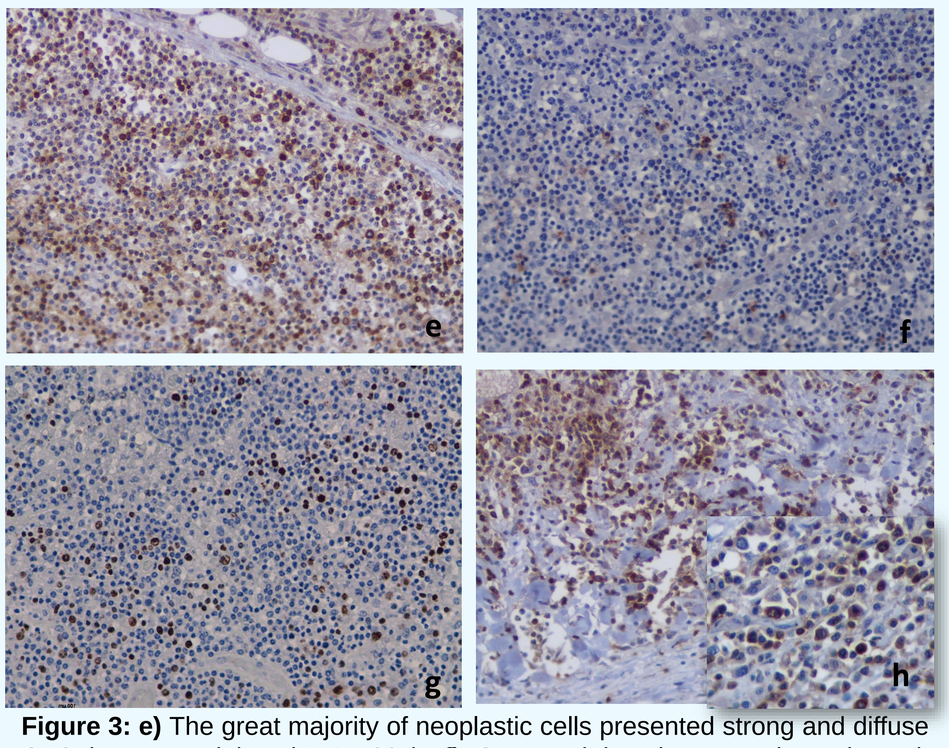

Figure 3: e) The great majority of neoplastic cells presented strong and diffuse CD3 immunostaining (DAB,100x); **f)** Scattered lymphocytes showed weak CD20 immunostaining; **g)** KI67 (DAB,100x); **h)** Neoplastic cells presente strong CD3 immunoexpression (DAB, 200x); inset: CD3-positive neoplastic lymphocytes (DAB, 600x).

Immunohistochemistry Results	
CD3	+++
CD79α, CD20	+
C-KIT, PD-L1, PAX-5	-
PI-KI67	19%

Legend: -, negative; +, weak immunostaining; ++, moderate immunostaining; +++, strong immunostaining; PI, proliferative index

EHV-5 PCR and sequencing analysis were negative.

DISCUSSION AND CONCLUSION

Equine lymphoma demonstrate unique and species-specifc characteristics, presenting mostly as a multi-organ disease that can be difficult to diagnose given the non-specificity of clinical signs [1]. The risk factors for the development of this disease are unknow. Nonetheless, a recent study by Miglio et al., (2019) described positivity for EHV type 5 in tissues with lymphoma. Histopathologically, equine lymphomas are generally heterogeneous, commonly presenting multinucleated giant cells, and previously associated with T-cell- derived lymphoma. Regarding IHC, according to WHO classification system, T-cell rich large B cell lymphoma (TCRBCL) is the most common phenotype in horses, characterized by the presence of T –lymphocytes, many of them reactive, among a small percentage of malignant B lymphocytes [2].

Based on these findings, a multicentric T lymphoma was diagnosed. There is still very little research regarding the molecular characterization of lymphoma in horses. As an entity itself quite heterogeneous, it is important to describe the interspecies particularities to understand its development and behavior.

REFERENCES:

1-Miglio, Arianna, et al. "Clinical and immunophenotypic findings in 4 forms of equine lymphoma." *The Canadian Veterinary Journal* 60.1 (2019): 33

2-Ness, SallyAnne L."Lymphoma" Equine Clinical Immunology, edited by M. Julia B. Felippe, 1st ed., John Wiley &Sons, Inc., Iwoa, USA, 2016, pp. 181-191