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Primary splenic diffuse large B-cell lymphoma with multinucleated giant cells in a horse

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Abstract: A diagnosis of a diffuse splenic large B-cell lymphoma with multinucleated giant cells in a 5-year-old mare was made based upon the clinical, pathological, and immunohistochemical findings. The enormous primary splenic mass weighed 51.75 kg. To the best of our knowledge, this is the biggest reported splenic mass and the first case of an equine diffuse large B-cell lymphoma with multinucleated giant cells.

Keywords: equine; immunohistochemistry; lymphoma; spleen

Lymphoma is the most common haematopoietic tumour in equids (Munoz et al. 2009). Conventionally, equine lymphoma is classified into generalised or multicentric lymphoma, alimentary lymphoma, cutaneous lymphoma, mediastinal or thoracic lymphoma and solitary lymphoma of extranodal structures (Taintor and Schleis 2011). Since there are no early pathognomonic signs, most cases are presented late in the course of the disease; early diagnosis is reliant upon the early detection of paraneoplastic signs, which are often overlooked (Taintor and Schleis 2011).

Primary splenic masses are infrequently encountered in horses and represent less than 1% of all diagnosed equine tumours (Sprayberry and Robinson 2014). Taintor and Schleis (2011) reported that the incidence of lymphoma in horses was approximately 1.3–2.8% of all diagnosed equine tumours (Taintor and Schleis 2011). In another study conducted on 203 cases of equine lymphoma, splenic lymphoma represented 2.5% of all lymphoma cases (Durham et al. 2013). Moreover, the most common lymphoma subtype was T-cell-rich large B-cell lymphoma (TCRLBCL) representing 43% of all diagnosed lymphoma cases (Durham et al. 2013)

Case description

A 5-year-old Arabian mare of an Egyptian line was referred to the Veterinary Health Centre at Jordan University of Science and Technology (VHC-JUST) with a history of weight loss and anoestrus. The mare had delivered a live foal 4 months earlier. According to the primary care veterinarian, no abnormality was noted during the pregnancy, parturition or postpartum periods. The mare lived in a farm with another 70 mares and 10 stallions of different ages. The mare was up to date with vaccination [equine rhinopneumonitis and equine influenza virus (Calvenza®-03EIV/EHV) and tetanus toxoid] and an anthelmintic treatment [ivermectin 1.2%, praziquantel 15% gel (Overmectina®)]. The diet had consisted of dry alfa-alfa (free choice), concentrate based on the body weight twice daily. Regular exercise to meet Arabian show horse requirements was carried out on all the horses which were managed in the same way.

Over the previous 4 months, the mare had been doing well clinically, but was anoestrous; this had been attributed to postpartum anoestrus due to nursing

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the foal. A full breeding soundness examination at VHC-JUST showed the mare to be bright, alert and responsive. Horse's temperature temperature, heart rate and respiratory rate were within the reference limits. A rectal palpation revealed a medial and caudal splenic displacement and splenomegaly.

The jugular blood was submitted for a complete blood count (CBC) including the differential counts. The abdominal fluid and ultrasound-guided aspirate from the abdominal mass were submitted for a cytological evaluation to evaluate the spleen. The erythrogram changes only showed a mild normocrocytic normochromic anaemia. The leukogram and thrombocytes were within the reference limits.

A rectal ultrasound using a liner 5 MHz probe revealed a splenic mass of mixed echogenicity. The splenic capsule was hyperechoic with an irregular appearance. The gigantic size of the mass and the machine settings prevented the full ultrasonographic size measurements of the spleen. No infectious agents or malignant processes were identified.

A fine-needle aspiration of the splenic mass was also submitted for a cytological examination. This was characterised by high cellularity. The cell population primarily consisted of a uniform population of large mononuclear cells with high nuclear to cytoplasmic ratios. The cells had rounded nuclei with finely stippled chromatin patterns and low amounts of pale blue cytoplasm and were intermixed with low numbers of binucleated and multinucleated giant cells. Many erythrocytes and amorphous necrotic debris were seen in the background. Based on these findings, the splenic mass was diagnosed as a malignant lymphoma.

Based on the diagnostic findings, an exploratory celiotomy was carried out. The spleen showed severe splenomegaly with diffuse splenic nodules. It occupied the entire left side of the flank. The rest of the abdominal viscera was unremarkable. Based on the findings, the owner elected for euthanasia.

At necropsy, a gigantic splenic mass was found occupying most of the abdomen. No other significant gross findings were present.

Grossly, the spleen was ovoid and totally obliterated by a solitary encapsulated whitish mass weighing 51.75 kg and measuring 53 × 45 cm with an irregular surface appearance with only a few areas of relatively normal splenic architecture (Figure 1). Upon dissection, the spleen was variegated with alternating foci of variably sized areas of haemorrhage, necrosis, oedema and neoplastic splenic tissue.

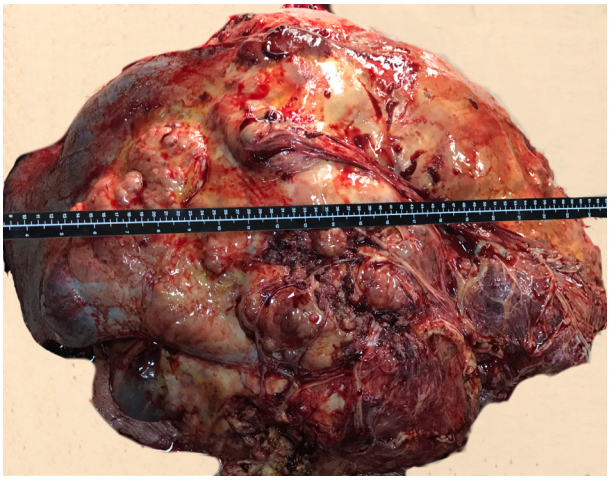

Figure 1. The spleen is ovoid and totally obliterated by a solitary encapsulated whitish mass weighing 51.75 kg and measuring 53 × 45 cm with a lumpy-bumpy appearance sparing a few areas of relatively normal splenic architecture

Multiple representative splenic sections were processed routinely from the formalinised tissue samples in an automatic processor and the haematoxylin and eosin (H&E) slides were prepared according to Suvarna et al. (2018).

Multiple sections were made from different areas of the mass which was encapsulated and composed of a neoplastic cell population effacing the normal architecture of the spleen (Figure 2A). These neoplastic cells formed a solid pattern of diffuse proliferation of a homogenous population of neo-

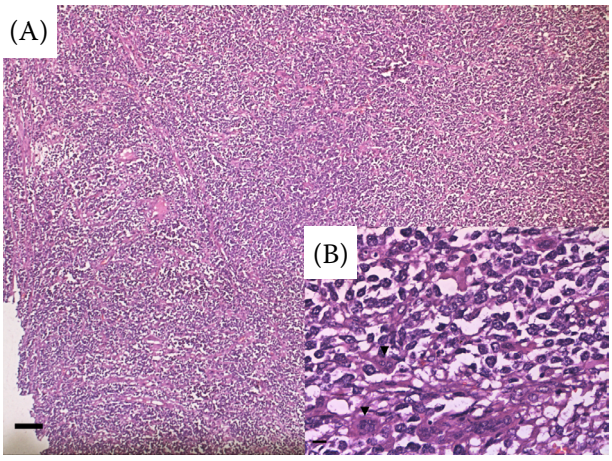

Figure 2. Histology

(A) Spleen, lymphoma. Neoplastic lymphocytes effaced the normal architecture of the spleen. H&E. Bar = 100 µm. (B) The inset is a higher magnification of (A). Note the arrow heads show multinucleated giant cells within the neoplastic lymphocytes. H&E. Bar = 10 µm

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plastic lymphocytes intermingled with frequent multinucleated foreign body giant cells and supported with fine fibrovascular stroma (Figure 2B). The neoplastic lymphocytes were medium to large, round to ovoid lymphocytes with an indistinct cell border with a uniformly low nucleus to cytoplasm ratio. They exhibited scant amounts of amphophilic cytoplasm and a single, round to ovoid nucleus with multiple nucleoli. The nucleus of tumour cells was large, being > 2 RBC's in diameter.

An immunohistochemistry with a labelled streptavidin-biotin (LSAB) method using a monoclonal antibody against B-cell antigen receptor B20 and a polyclonal antibody against pan T-lymphocytes markers (CD3) were carried out. In some sections, multiple variably sized areas of severe necrosis, fibrinous oedema and haemorrhage were present. Immunohistochemically, the neoplastic lymphocytes were strongly positive to anti CD20 (Figure 3) and negative to anti-CD3 (Figure 4). The multinucleated giant cells, throughout the examined sections, were negative for both CD20 and CD3.

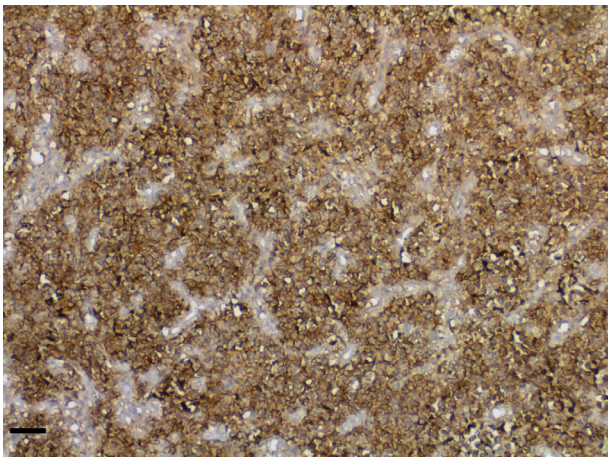

Figure 3. Immunohistochemistry. Spleen, lymphoma. Immunohistochemical labelling for CD20

The neoplastic cells have strong cytoplasm CD20 labelling. Immunolabelling with anti-CD20, the streptavidin-peroxidase method, diaminobenzidine (DAB) substrate, haematoxylin counterstain. Bar = 50 μm

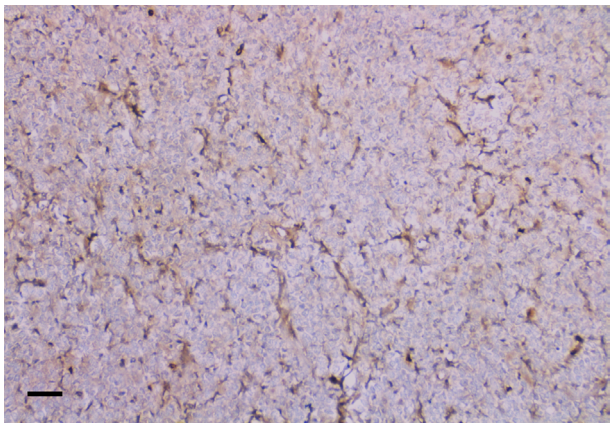

Figure 4. Immunohistochemistry. Spleen, lymphoma. Immunohistochemical labelling for CD3

The neoplastic cells are CD3 negative. Immunolabelling with anti-CD3, the streptavidin–peroxidase method, DAB substrate, haematoxylin counterstain. Bar = 50 μm

DISCUSSION AND CONCLUSIONS

In the present case, the pathological and immunohistochemistry findings indicated that the mass was composed of a homogenous population of neoplastic lymphocytes of B-cell origin. However, the origin of the foreign body multinucleated giant cells could not be determined. They were negative for CD20 and CD3 immunostaining. Moreover, there were no accompanying infectious processes that would explain the presence of these multinucleated giant cells.

A B-cell lymphoma with multinucleated giant cells was previously described in a cat (Ohshima et al. 2004). In that case, the B-cell lymphoma was of an immunoblastic type, but in the present case, the lymphoma was of a diffuse large B-cell type. Furthermore, in the feline case, the origin of multinucleated giant cells was not determined since they were immunohistochemically negative for both pan B- and T-cell markers, as was the result in the present case. Previously, it was reported that Langhans-type multinucleated giant cells were consistently present in equine lymphoma as a response to the interferon gamma produced by the neoplastic T-cell lymphocytes (Meyer et al. 2006). In the present case, only foreign body multinucleated giant cells were seen throughout the examined tissue sections and the neoplastic cells were of a B-cell origin.

A primary splenic lymphoma of B-cell origin was diagnosed in a 4-year-old thoroughbred stallion without invasive growth or metastasis (Tanimoto et al. 1994). The splenic mass was solitary, measured 20 × 20 × 15 cm and contained haemorrhagic and necrotic foci (Tanimoto et al. 1994). In the current case, the mass was primarily in the spleen and no other masses were present within the abdominal viscera and the mass was gigantic. A similar diagnosis of a diffuse large B-cell lymphoma was reported in a 13-year-old Hirsh gelding horse (Miglio

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et al. 2019). However, in that case, no multinucleated giant cells of a similar type to those identified in the present case were reported.

To the best of the authors' knowledge, this is the first case of a primary gigantic splenic B-cell lymphoma in a horse with no apparent metastasis. A primary splenic B-cell lymphoma should be included in the differential diagnosis of splenomegaly.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Durham AC, Pillitteri CA, San Myint M, Valli VE. Two hundred three cases of equine lymphoma classified according to the World Health Organization (WHO) classification criteria. Vet Pathol. 2013 Jan;50(1):86-93.

Meyer J, Delay J, Bienzle D. Clinical, laboratory, and histopathologic features of equine lymphoma. Vet Pathol. 2006 Nov;43(6):914-24.

Miglio A, Morelli C, Gialletti R, Lauteri E, Sforna M, Marenzoni ML, Antognoni MT. Clinical and immunophenotypic findings in 4 forms of equine lymphoma. Can Vet J. 2019 Jan;60(1):33-40.

Munoz A, Riber C, Trigo P, Castejon F. Hematopoietic neoplasias in horses: Myeloproliferative and lymphoproliferative disorders. J Equine Sci. 2009;20(4):59-72.

Ohshima M, Morita T, Sawada M, Shimada A, Kobayashi H, Sawashima K. B-cell immunoblastic lymphoma with multinucleated giant cells in a cat. J Vet Med Sci. 2004 Feb; 66(2):189-91.

Sprayberry KA, Robinson NE. Robinson's current therapy in equine medicine. St. Louis, Missouri: Elsevier Health Sciences; 2014. 998 p.

Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Amsterdam: Elsevier Health Sciences; 2018. 672 p.

Taintor J, Schleis S. Equine lymphoma. Equine Vet Educ. 2011;23(4):205-13.

Tanimoto T, Yamasaki S, Ohtsuki Y. Primary splenic lymphoma in a horse. J Vet Med Sci. 1994 Aug;56(4):767-9.

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