

B-cell uterine lymphoma in a mare with infertility

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Summary

The article presents a case of B cell lymphoma affecting the uterus and spleen in a mare delivered for veterinary examination due to infertility. According to information obtained from the animal's owner, the mare did not become pregnant despite mating in the last two reproductive seasons. Apart from infertility, the owner did not notice any other problems with the mare's health. The mare was in good body condition and physical examination revealed no significant abnormalities except scant, serosanguineous vaginal discharge. Rectal palpation of the reproductive tract showed multiple nodular masses located in both uterine horns. Transrectal ultrasonography confirmed the presence of well circumscribed, hyperechoic masses of various sizes (with a diameter of 2 to 6 cm) in the uterine wall protruding into the uterine lumen. Moreover, small amounts of fluid in the uterus were found. Routine hematological and biochemical blood tests showed mild anemia and elevated alkaline phosphatase. Based on these findings, the first diagnosis of uterine neoplasia was made. Therefore, a decision was made to perform a hysterectomy. During laparotomy, in addition to the affected uterus, a very large splenic tumour was found. In view of clinical diagnosis of probable metastatic neoplasia and poor prognosis the mare the owner agreed to euthanize the mare. Post-mortem examination revealed numerous different-sized (with the largest diameter of 6 cm), well circumscribed, firm, gray to white nodular masses expanding the wall of the uterus and protruding into its lumen and a very large irregularly shaped tumour, 30 cm in diameter, in the spleen. On cross-sectional surfaces, masses in both the uterus and the spleen were white with small foci of extravasation. Lymph nodes and other organs showed no evidence of neoplasia. Histological examination of collected masses and immunohistochemical staining showed multicentric diffuse large B-cell lymphoma involving the uterus and spleen.

Keywords: B-cell lymphoma, uterus, mare

Lymphoma, also known as lymphosarcoma or malignant lymphoma, although generally rare in horses, it is the most common hematopoietic neoplasia in this species (13, 20). Literature data indicate that the frequency of this disease in the equine population is 0.002-0.5%, and the overall incidence of lymphoma is approximately 1.3-2.8% of all equine tumours (20). There is no apparent breed or sex predilection and any age of horse can be affected, although a greater predisposition for horses within 5-10 years of age is reported (7, 13). The pathogenesis of equine lymphoma is still poorly understood. The possible involvement of herpesvirus infections in neoplastic transformation has been speculated (14, 22).

Clinical signs of lymphoma in horses are nonspecific and depend on the organs affected. The most common clinical signs of equine lymphoma include weight loss, depression, lethargy, swelling of the ventral body wall

or distal limbs, recurrent fever, and lymphadenopathy. Because the clinical signs of lymphoma typically develop insidiously, diagnosis of lymphoma for most affected horses is made in the late phase of the disease (7, 20).

The diagnosis of equine lymphoma involves physical examination, including the evaluation of superficial and deep lymph nodes, *per rectum* palpation of abdominal organs, and ultrasound examination. Definitive diagnosis is made through the identification of neoplastic lymphocytes in tissues, fluids or peripheral blood. Histopathological examination together with immunohistochemical examination remains the most accurate method for diagnosing this disease (7, 13, 18, 20).

The prognosis of lymphoma in horses is poor and euthanasia is usually the only reasonable option. To prolong the survival time, surgical excision, radia-

tion, and chemotherapy are available as therapies in horses (20).

Based on anatomical location, equine lymphoma can be classified as multicentric, alimentary, mediastinal, cutaneous, and solitary (extranodal) (7, 14, 20). The multicentric lymphoma, affecting the lymph nodes and/or many different organs, is the most frequent in horses, followed by the alimentary form (7, 14). Based on morphology and immunophenotype, various subtypes of equine lymphoma are distinguished, including B or T cell lymphoma and T-cell-rich large B-cell lymphoma (3, 7, 10). According to the study by Durham et al. (7), conducted on 203 cases of equine lymphoma, T-cell-rich large B-cell lymphoma (TCRLBC) is the most common lymphoma subtype in horses. Lymphoma very rarely affects the uterus of mares (4, 6, 8). This case report describes a case of multicentric B cell lymphoma involving the uterus and spleen in the mare delivered for veterinary examination due to infertility.

Case description

The 14-year-old mare was delivered to the Department and Clinic of Animal Reproduction in Lublin for reproductive examination due to infertility. According to information obtained from the animal's owner, the mare had given birth several times in the past; however, she did not become pregnant despite mating in the last two reproductive seasons. Apart from infertility, the owner did not notice any other problems with the mare's health. The mare was in good body condition and physical examination revealed no significant abnormalities, except scant, serosanguineous vaginal discharge. The vital parameters were within normal limits. Rectal palpation of the reproductive tract showed an enlarged uterus and multiple nodular masses located in both uterine horn. Transrectal ultrasonography confirmed the presence of well circumscribed, hyperechoic masses of various sizes (with a diameter of 2 to 6 cm) in the uterine wall protruding into the uterine lumen. Moreover, small amounts of fluid in the uterus was found. Routine hematological and biochemical blood tests showed mild anemia (red blood cells – $4.9 \times 10^6/\mu\text{L}$; hemoglobin –

7.49 g/dL; white blood cells – $9.93 \times 10^3/\mu\text{L}$) and elevated alkaline phosphatase (294 U/L). Based on these findings, the first diagnosis of uterine neoplasia was made. Therefore, a decision was made to perform a hysterectomy. During laparotomy, in addition to the affected uterus, a very large splenic tumor was found. In view of clinical diagnosis of probable metastatic neoplasia, and poor prognosis the owner agreed to euthanize the mare.

Post-mortem examination revealed numerous different-sized (with the largest diameter of 6 cm), well circumscribed, firm, gray to white nodular masses expanding the wall of the uterus and protruding into its lumen, and a very large irregularly shaped tumour, 30 cm in diameter, in the spleen. On cross-sectional surface, masses in both the uterus and the spleen were white with small foci of extravasation (looked similarly) (Fig. 1). Lymph nodes and other organs showed no evidence of neoplasia. In addition, a moderate invasion of *Gasterophilus intestinalis* was found in the mucosa of the gastric cardia.

Representative samples of the masses located in the uterus and spleen were collected for histopathological examination, fixed in 10% neutral buffered formalin, embedded in paraffin, sliced on a sledge microtome into 4 μm thick sections and stained with hematoxylin and eosin (HE). Histologically the submucosa, superficial endometrial stroma and uterine myometrium were multifocally to coalescent expanded by sheet-like proliferation of large neoplastic lymphoid cells supported by scant fibrovascular network. Neoplastic cells had scant eosinophilic cytoplasm with distinct margins and round-to-oval, large nuclei with vesicular chromatin and 1 or 3 prominent nucleoli. There was moderate anisocytosis with cells varying from approximately 10-20 μm in diameter. The mitotic count was 12 in 2.37 mm^2 . Small numbers of pyknotic neoplastic cells and small lymphocytes were scattered throughout the examined sections. The overlying endometrial epithelium was flattened, with attenuated glands and compressed by the neoplastic infiltrate. The samples from the spleen were characterized by a similar histological pattern consistent with lymphoma. To determine the origin of neoplastic cells, sections were labeled immunohistochemically with a panel of antibodies for B- and T-cell antigens following a routine

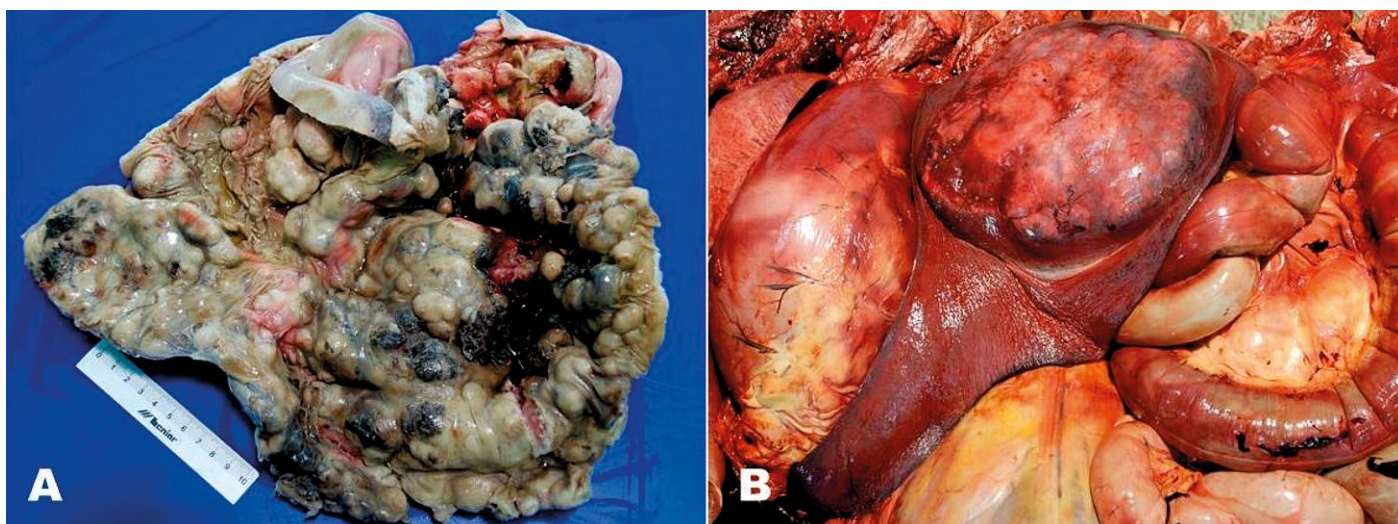


Fig. 1. Macroscopic view of the uterine lymphoma (A) and splenic lymphoma (B)

protocol. Briefly, 4 μm thick tissue sections were applied to Super Frost slides (Menzel-Glaser) and then incubated at 56°C for 12 hours. After dewaxing in xylene and rehydra-

tion in decreasing concentrations of alcohols and distilled water section were immersed in a 0.3% hydrogen peroxide solution for 15 minutes to block endogenous peroxidase.

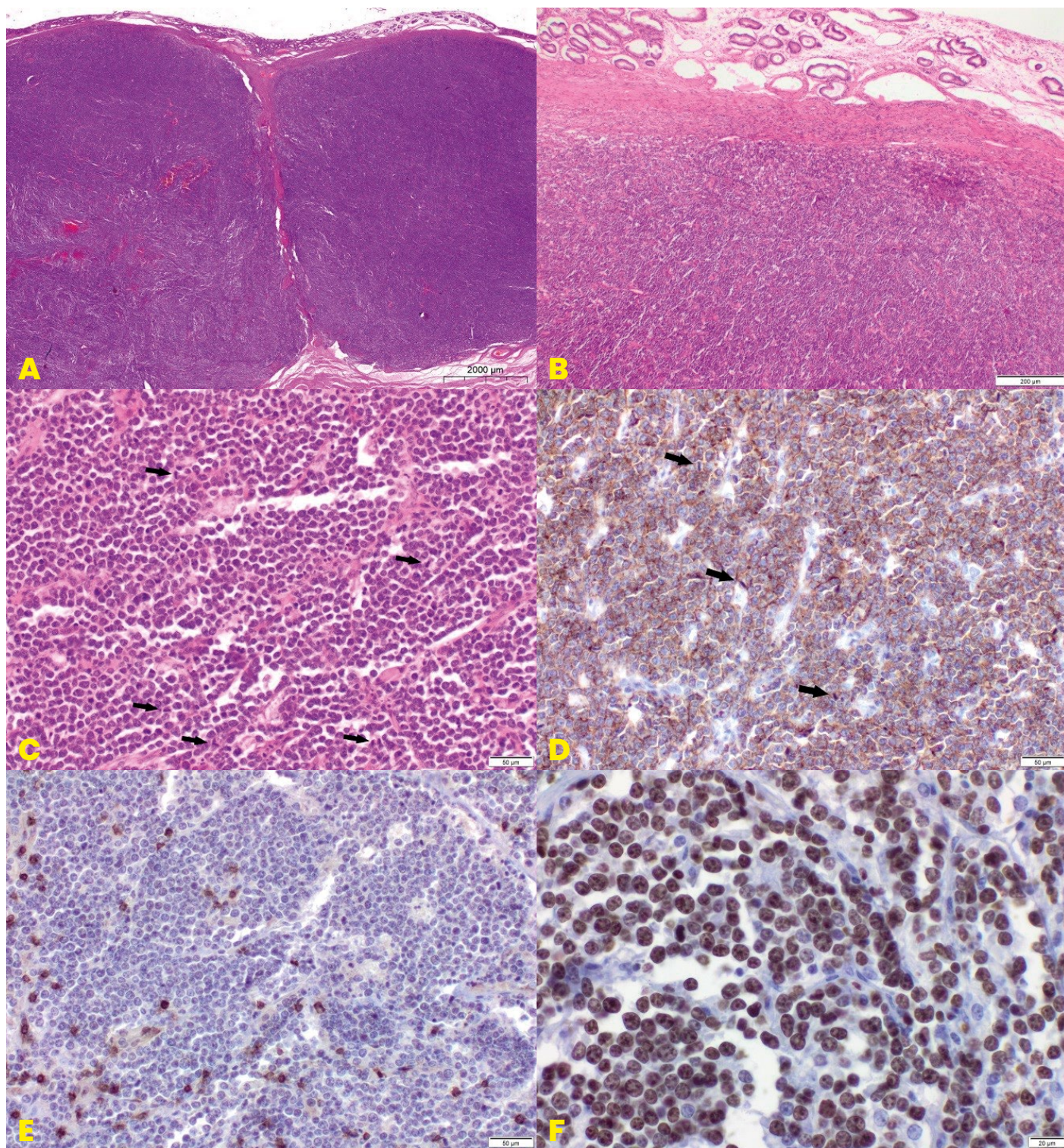


Fig. 2. Histopathological picture of the uterine lymphoma

Explanations: A – Partially encapsulated neoplastic masses composed of a dense population of round cells that efface and expand the uterine submucosa and myometrium. HE. Bar = 2000 μm . B – Dense sheets of large neoplastic lymphocytes under attenuated endometrium. HE. Bar = 200 μm . C – Diffuse large B-cell lymphoma, large neoplastic lymphocytes with round-to-oval nuclei (nuclei approximately equal to 2 $\times$ the diameter of an RBC), prominent nucleoli and scant cytoplasm. Numerous mitotic figures are noted (arrows). HE. Bar = 50 μm . D – The majority of neoplastic cells express strong, positive immunostaining on the cytoplasmic border for CD20 antibody, indicating that these cells are B lymphocytes. Note numerous cells with mitotic figures (arrows). Immunohistochemistry (IHC), Mayer's hematoxylin counterstain. Bar = 50 μm . E – Scattered, reactive lymphocytes express membrano-cytoplasmic CD3 immunostaining. Immunohistochemistry (IHC), Mayer's hematoxylin counterstain. Bar = 50 μm . F – Positive Pax5 immunostaining in the nuclei of B-cells. Immunohistochemistry (IHC), Mayer's hematoxylin counterstain. Bar = 20 μm

Incubation with primary antibodies was carried out at 37°C in a humidity chamber for 60 minutes. The following primary antibodies were used in the study: anti-CD3 (polyclonal rabbit anti-human CD3, A0452; DAKO, Glostrup, Denmark, diluted 1:50), anti-CD79a (monoclonal mouse anti-human CD79a, clone HM57, Dako, Glostrup, Denmark diluted 1:100), anti-CD20 (polyclonal rabbit anti-human CD20, RB-9013-P; Thermo Fisher Scientific, Waltham, MA, U.S., diluted 1:200) and anti-Pax5 (monoclonal mouse anti-human Pax5, clone 1EW, Leica Biosystem, Nussloch, Germany, diluted 1:100). Heat-induced antigen retrieval was performed in a specific buffer with proper pH, followed by cooling for 20 min at room temperature. For immunohistochemical examination, a system for detection of antigen-antibody complexes Ultra Vision Quanto Detection System HRP DAB was used (Epredia, Breda, Netherlands). The enzyme labeling the reaction site was horseradish peroxidase conjugated with streptavidin and tetrahydrochloride-3'-3'-di-aminobenzidine (DAB) used as a chromogen (SK-4100, Vector Laboratories, Peterborough, UK). Tissue sections were counterstained with Mayers' hematoxylin and covered with PERTEX (Histolab). In all immunohistochemical reactions, a double-control system was used: a negative control in which the incubation with the primary antibody was replaced with appropriate IgG sera and a positive control in which the incubation was carried out on normal horse lymph node. The neoplastic cells were strongly positive for CD79a, CD20 and Pax5 (B-cell markers) and negative for CD3 (T-cell marker) (Fig. 2). Moderate numbers of scattered small round cells stained positive for CD3. This was interpreted as infiltration of mature T-cells within the neoplastic B-cell population. Since most of the lymphoid cells in the examined uterine and splenic masses express B-cell-surface or nuclear molecules a diagnosis of multicentric diffuse large B-cell lymphoma (DLBCL) involving the uterus and spleen was made.

Discussion

The most common form of lymphoma in horses is multicentric lymphoma, which is characterized by widespread involvement of lymph nodes and/or a variety of organs most likely through distribution of neoplastic lymphocytes via lymphatic circulation (20). Liver, spleen, intestine, kidney and bone marrow are the organs most commonly affected in equine multicentric lymphoma (6, 13, 20). Various locations of solitary lymphoid tumours have been reported in horses, including the spleen, nasopharynx, sinus, tongue, meninges and pelvis (14, 15, 20, 21). An extremely rare location for lymphoma in horses is the uterus. In the case described here, a multicentric form of lymphoma involving the uterus and spleen was diagnosed. To the author's knowledge, multicentric lymphoma located in the uterus and spleen has not been previously reported in horses. There are only a few reports in the available literature about uterine lymphoma in mares (4, 6, 8). In all these cases, the uterus was one of organs involved in multicentric forms of lymphoma. In the case presented here, uterine lymphoma was characterized by

the presence of numerous tumours of various sizes in uterine wall. Freeman et al. (8) described case of uterine lymphoma in a 22-year-old mare with multicentric lymphoma. In their case, rectal and ultrasound examinations confirmed postmortem examination revealed a large uterine mass (approximately 20-30 cm in size) located in the left uterine horn and the lumen of the uterus was continuous throughout the mass. There was also fluid accumulation in the uterus. Moreover, postmortem examination showed generalized lymphadenopathy affecting the bronchial, cranial mediastinal, gastric, omental and sublumbar lymph nodes. Canisso et al. (4) reported the multicentric lymphoma involving the uterus, lymph nodes, right ovary, kidneys, and lungs in 5-year-old mare. Those authors found an enlarged uterus with thickened uterine wall (> 3 cm) and containing a moderate amount of fluid. Also the case report by Claes et al. (6) concerns multicentric lymphoma involving the uterus. In an 8-year-old mare these authors detected a firm, well circumscribed, 5 × 5 cm in size, irregular shape mass located near the tip of the left uterine horn. In addition to the uterus, the left kidney, spleen, left adrenal gland and lymph nodes were involved.

In our case, in addition to the uterus, the spleen was also affected by the lymphoma. The solitary form of splenic lymphoma is rare in horses (5, 14, 21), and generally, the spleen is affected in the course of the multicentric form of lymphoma (6, 8, 20). Typically, the splenic lymphoma is characterized by multiple, moderately firm, variably sized nodules throughout the splenic parenchyma (5, 8). In this case, similarly to the cases described by Tanimoto et al. (21) and Miglio et al. (14), the spleen was affected by a large, well-defined solitary mass.

Characteristically, despite the extensive involvement of the uterus and spleen by lymphoma there were no significant changes in the mare's health. This is consistent with previous reports by other authors (14). Clinical symptoms of equine lymphoma are not pathognomonic and vary depending on the organs affected and the stage of disease. Nonspecific clinical signs such as weight loss, anorexia, edema, and lethargy are often observed (3, 5, 6, 13). Signs specifically referable to uterine lymphoma in mare are relatively very rare. Claes et al. (6) observed in a mare with uterine lymphoma scanty sanguinous vaginal discharge. This symptom also occurred in our case.

In our case, the mare was submitted for treatment due to infertility. Various pathological masses in the uterus, although rare in mares, may have a negative impact on their reproduction (12, 17). The most frequently reported uterine mass in mares is a leiomyoma, a benign tumour arising from smooth muscle (12, 17). In contrast, uterine lymphoma is extremely rare and its impact on mare's reproduction is very poorly described. In the available literature, one report indicates uterine lymphoma as the probable cause of abortion

in a mare (4). In the presented case, the time of onset of the neoplastic lesions in the uterus was unknown. Therefore, we can only speculate that the mare's unsuccessful mating in the last two breeding seasons was caused by the uterine lymphoma.

It is difficult to determine in our case, where the neoplastic process started: in the uterus or in the spleen. Uterine mucosa contains lymphoid tissue, which may undergo neoplastic transformation and then metastasize to other organs (11). Primary uterine lymphoma have been found in female cats (1) and bitches (9, 11, 16). As indicated in previous reports, the uterus may also be a site of metastasis (4, 6, 8).

Uterine masses in mares can be detected during palpation *per rectum* of the reproductive tract, transrectal ultrasonography or hysteroscopy; however, the diagnosis of uterine lymphoma requires histopathological examination (4, 6, 8). To characterize the histogenesis and the immunophenotype of the neoplastic cells, immunohistochemistry must be performed. In our case, the CD3 was used as T-cell marker and the CD20, Pax-5 and CD79 α were selected as B-cell markers. CD20 has better labeling quality in equine lymphocytes as previously reported by Durham et al. (7). Pax-5, as a new B-cell marker showing a nuclear reaction is now increasingly used in the differential diagnosis of lymphomas in animals (2, 19). In our case, similarly to the reports by Canisso et al. (4) and Claes et al. (6), histopathological and immunohistochemical findings identified uterine B-cell lymphoma. In the case described by Freeman et al. (8), uterine T cell lymphoma in a mare was diagnosed.

Results of laboratory tests in horses with lymphoma are inconclusive and they may vary from case to case. The most common hematological and biochemical abnormalities in horses with lymphoma involve anemia, thrombocytopenia, leukemia, neutrophilia, hyperfibrinogenemia, hypoalbuminemia, and hyperglobulinemia (8, 13, 20). In our case, routine hematological and biochemical examination revealed anemia and an increased level of alkaline phosphatase. These abnormalities were found in mares with lymphoma by other authors (8, 14). Unfortunately, in the presented case, fibrinogen, albumin and globulin levels were not examined.

In our case, the first diagnosis was a primary uterine neoplasm which was why the decision was made to perform hysterectomy. However, laparotomy revealed the involvement of the spleen and suggested metastatic neoplasia. In such a situation, the mare was euthanized due to the poor prognosis. It is suggested that in a case of primary equine uterine lymphoma hysterectomy could be an appropriate form of therapy (6). However, in horses with multicentric lymphoma, regardless of lymphoma subtype, the prognosis is poor and most horses with this form of lymphoma die or are euthanized within a few months after diagnosis (4, 6, 8, 13, 18).

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