Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #: **214**; Month: **June**; Year: **2023**

Answer Sheet

Title: Enzootic bovine leukosis in a cow.

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Clinical History: A 5-year-old, Jersey cow was evaluated by a veterinary practitioner for a 30-day history of unilateral exophthalmos (Fig. 1). After 15 days, the cow presented lameness followed by progressive weight loss and pelvic limbs paresis, culminating in persistent sternal recumbency (Fig. 2). The superficial inguinal lymph nodes were enlarged. Due to the poor prognosis, the cow was euthanized and submitted to a postmortem examination.

Gross Findings: The cow was in poor body condition with mild amounts of subcutaneous and visceral fat stores. The oral and conjunctival mucous membranes were pale. There was severe exophthalmos in the right eye, caused by a soft, homogenous white to yellow mass (6 cm in diameter) (Fig. 3) in the retrobulbar space. Similar irregular masses were seen in the left renal pelvis, partially effacing the renal parenchyma, and in the epidural space, circumferentially surrounding the pachymeninges (extradural location) (Fig. 4) of the lumbar segment of the spinal cord. The superficial inguinal (supramammary) lymph nodes were markedly enlarged and, on the cut surface, had homogenous white to yellow discoloration and loss of the corticomedullary junction. Multifocal areas of the abomasum wall were moderately thickened and expanded by a soft, homogenous white to yellow masses. No significant alterations were observed in other organs.

Gross and Microscopic Images:

Figure 1. Cow, enzootic bovine leukosis. Anterior protrusion of the right eye out of the orbit (unilateral exophthalmos).

Figure 2. Cow, enzootic bovine leukosis. Sternal recumbency and pelvic limbs caudally extended.

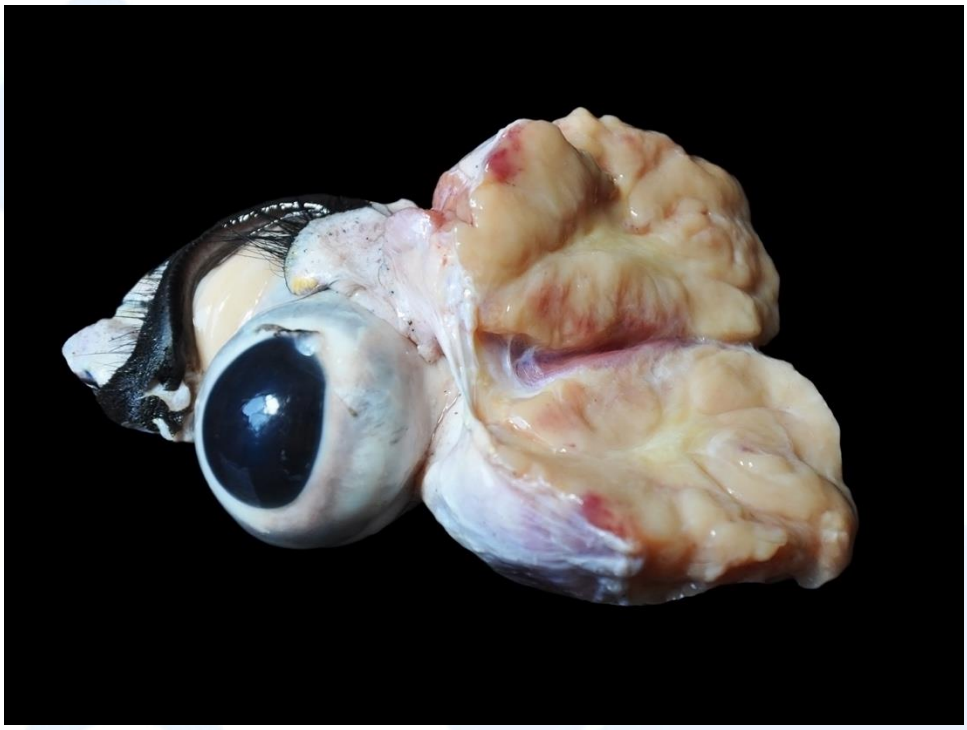

Figure 3. Cow, enzootic bovine leukosis. Cut surface of the mass in the retrobulbar space.

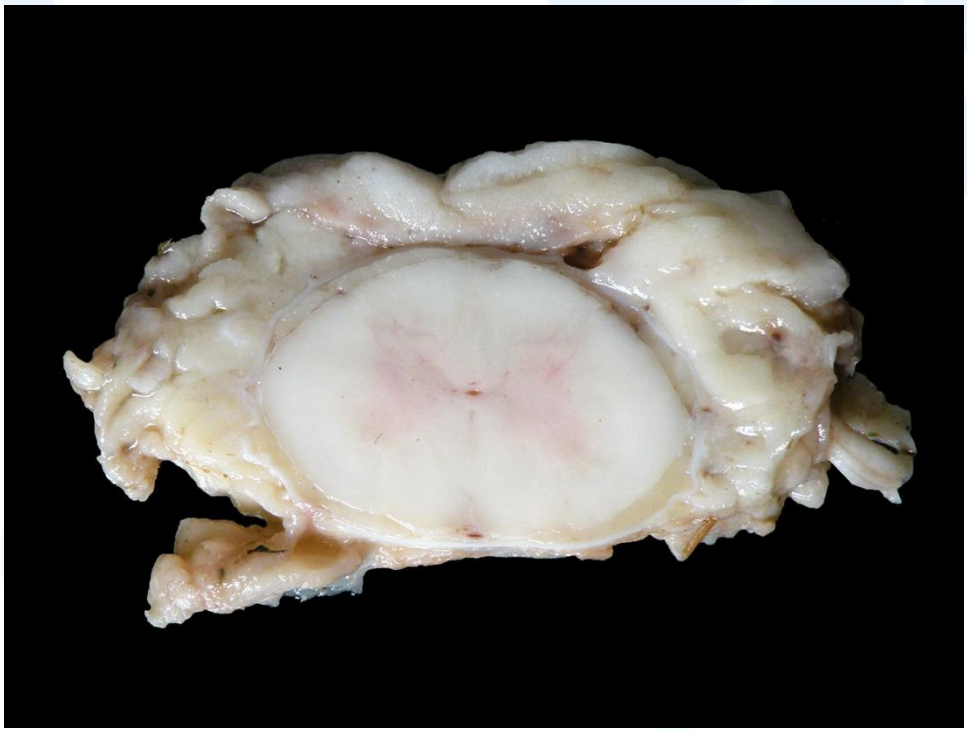

Figure 4. Cow, enzootic bovine leukosis. Irregular masses in the epidural space, peripheral to the pachymeninges, of the lumbar segment of the spinal cord.

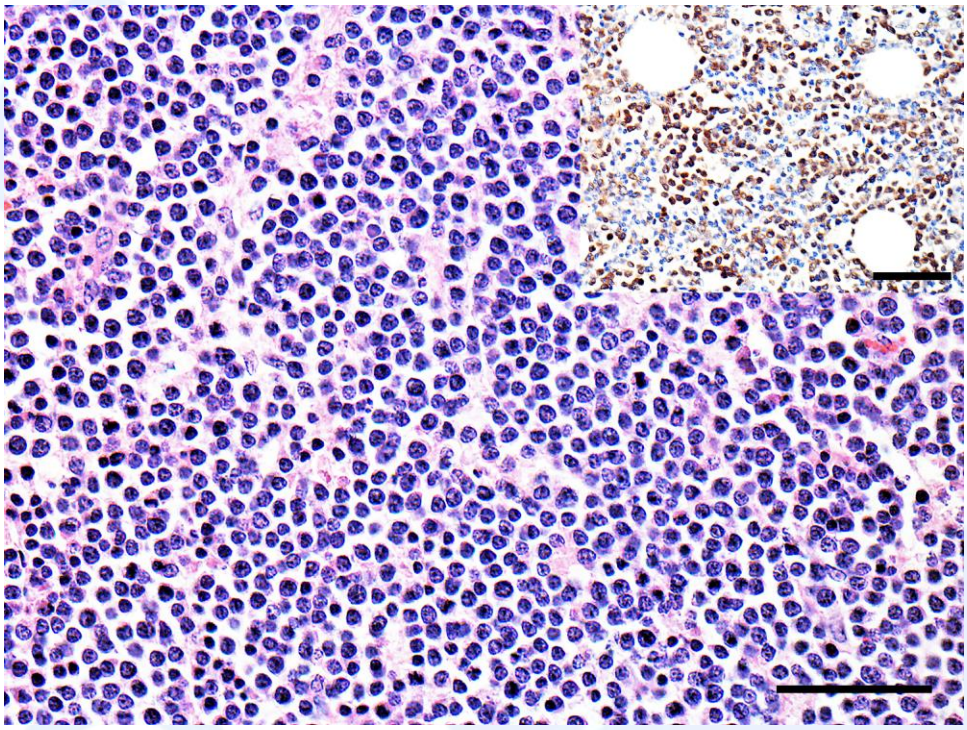

Figure 5. Cow, enzootic bovine leukosis. Nodal mass. Sheet of large neoplastic lymphocytes. H&E, bar = 200 µm. Inset: Extradural mass. Diffuse, intense cytoplasmatic immunoreactivity for CD79α in the neoplastic cells. IHC, bar = 200 µm.

Histological Description: The retrobulbar, extradural, renal, nodal, and abomasal masses consisted of a nonencapsulated, densely cellular, poorly demarcated neoplastic proliferation of large lymphocytes (more than 14 µm in size) arranged in sheets within scant amounts of fibrous stroma (Fig. 5). Neoplastic cells had distinct cell borders and mild amount of pale eosinophilic cytoplasm. Nuclei were large and round with coarsely granular chromatin and a single large central nucleolus. Anisocytosis and anisokaryosis were moderate. There were five mitotic figures per 2.37mm². Within of the neoplastic proliferation, there were multifocal areas of necrosis (<10% of the evaluated sections).

Immunohistochemical and Molecular Findings: For diagnostic confirmation and neoplastic cell immunophenotyping, sections of the extradural mass were submitted to immunohistochemistry (IHC) using anti-CD79α and anti-CD3 antibodies. Diffuse, intense cytoplasmatic immunoreactivity for CD79α was seen in the neoplastic cells (inset of Fig. 5). Neoplastic cells were not immunoreactive for CD3.

Morphologic Diagnosis: Lymphoma.

Classification: Diffuse large B-cell lymphoma.

Molecular Findings: Fresh samples of the extradural mass were sent for additional molecular testing. A 446 bp fragment of the *env* gene of the bovine leukemia virus (BLV) was amplified by RT-PCR, using OBLV1A F and OBLV6A R primer pairs.

Etiological Agent: BLV.

Name of the Condition: Enzootic bovine leucosis (EBL).

Probable Pathogenesis Pathway:

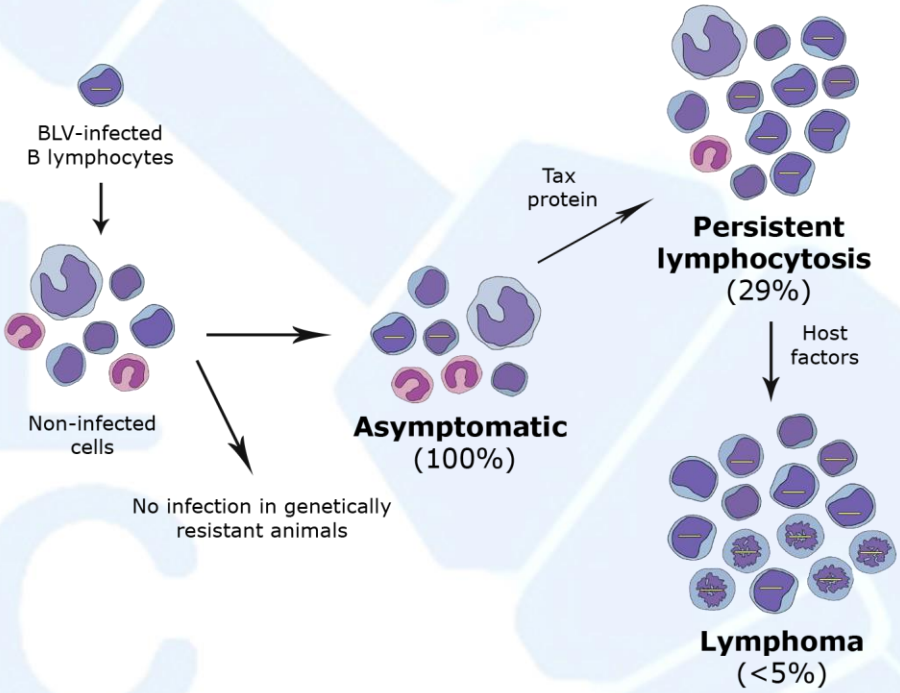

Figure 6. Schematic representation of the probable pathogenesis pathway of BLV-induced lymphoma, classically divided into three progressive stages: **Asymptomatic**. Infection of T lymphocytes, B lymphocytes, monocytes, and granulocytes following exposure to BLV-infected B lymphocytes. **Persistent lymphocytosis**. Immortalization of IgM+ CD5+ B lymphocytes, mediated by BLV Tax protein, and polyclonal proliferation. **Lymphoma**. Monoclonal proliferation and malignant transformation of B lymphocytes with involvement of host factors. The percentage represent the number of seroconverted cattle that develop each disease stage.

Comments: Lymphoma, also called lymphosarcoma and malignant lymphoma, is a common lymphoid neoplasm in cattle (6). Bovine lymphoma is classically classified by the frequency of occurrence into EBL and sporadic bovine lymphoma (SBL). The SBL is further subdivided according to the age at onset and organ/system involved into juvenile multicentric, cutaneous, and thymic (8). EBL accounts for more than 95% of the cases of lymphoma in cattle and is caused by BLV, an enveloped RNA virus member of the Retroviridae family and Deltaretrovirus genus (8). Although the infection with BLV is widespread on all continents, some countries have implemented effective eradication programs (2). The occurrence of lymphoma, the decreased productivity in clinical and subclinical animals, and the restrictions on international commerce result in significant economic losses, especially in herds with a high rate of infection (2). Transmission occurs horizontally via the transfer of BLV-infected lymphocytes from one animal to another (7). Important sources of infection include contaminated surgical instruments (eg, needles and ear tattooing pliers), blood

transfusions, and vaccines containing blood (2). Transmission from parturition, rectal palpation, and blood-sucking insects can also occur (2).

BLV infection alone is not enough for lymphomagenesis in cattle, and some subsequent events can be involved in the process (1). Although not entirely clear, the probable pathogenesis pathway of EBL may be linked to gene transactivation and is classically divided into three stages (1,2). Regardless of the route of infection, exposure to BLV-infected B lymphocytes results in the establishment of a permanent infection in nonspecific cells with the development of detectable antibodies and without clinical signs/laboratory abnormalities (asymptomatic stage, also called aleukemic infection) (1,2). Some cattle fail to become infected likely due to genetic resistance (2). After the asymptomatic stage, BLV Tax protein immortalizes part of BLV-infected cells (presumably IgM+ CD5+ B lymphocytes) and causes their polyclonal proliferation (persistent lymphocytosis stage) (8). This process is considered the first step of the neoplastic transformation (2); however, the subsequent involvement of host factors, such as p53 mutation, genetic variation in the bovine leukocyte antigen, and a polymorphism in the promoter region of the tumor necrosis factor-α gene, is crucial for monoclonal proliferation and malignant transformation of B lymphocytes (lymphoma stage) (1).

Lymphoma, the only clinically apparent form of EBL, usually occurs in cattle over two years of age, especially between 5 and 8 years (2). Lesions are more common in dairy cattle compared to beef cattle due to husbandry practices and the longest life-span (8). The clinical signs are variable and unspecific, such as body weight loss, inappetence, pallor, weakness, and decrease in milk production (2). However, specific clinical syndromes may also develop according to the site affected by the lesions, including enlargement of peripheral and visceral lymph nodes, tachypnea, stertorous respiration, dyspnea, subcutaneous edema, dysphagia, diarrhea, indigestion, tympanums, congestive heart failure, decubitus, hindlimb lameness, and paraplegia (4,5,8). Although the time between the onset of clinical signs and death varies (5), most cases tend to have subacute to chronic course (2). Few affected cattle have a peracute course, which is characterized by sudden death due to abdominal hemorrhage caused by splenic rupture or generalized peritonitis from perforated abomasal ulcer (2). Moreover, hematological assessment can reveal anemia, leukocytosis, and reactive lymphocytosis (2).

At postmortem examination, EBL appears as soft, white to yellow tumors in any organs, especially superficial and visceral lymph nodes, heart, abomasum, intestine, kidneys, and spinal cord (5,8). Affected lymph nodes have enlarged parenchyma and loss of corticomedullary junction (5). In the heart, masses or infiltrative tumors are mainly seen in myocardium, pericardium, and subepicardial tissue of right atrium; these cases can result in right-sided congestive heart failure with hydrothorax, ascites, subcutaneous edema, and nutmeg liver (2). Abomasal lesions are characterized by irregular thickening or masses in the submucosa, occasionally with obstruction or mucosal ulceration (8). Similar lesions can also be found in the intestinal wall (2). Involvement of kidneys usually includes enlargement of the parenchyma and diffuse masses; renal lesions coming from the renal pelvis may be associated with hydronephrosis. Spinal lesions form irregular tumors in the epidural space, peripheral to the pachymeninges (extradural), mainly of the lumbosacral segment (4). Frequently, the distinction between spinal tumors and perineural fat can be difficult (8). Uterus, retrobulbar space, omasum, rumen, reticulum, and skeletal muscle are also affected (8).

EBL is histologically characterized by sheets of neoplastic lymphocytes distorting or effacing the normal architecture of the affected tissue/organ (5). As lymphomas comprise a heterogeneous group of malignant neoplasms arising from lymphoid tissue with different clinical manifestations and cytopathologic findings, several classification systems have been proposed over the years (3,5). According to the criteria of the National Cancer Institute Working Formulation for human non-Hodgkin's lymphomas, which identifies subtypes based on their histomorphology into low, intermediate, and high-grade groups (3), most of the BLV-induced lymphomas are the cleaved variant of the diffuse large cell type with high mitotic index (5,10). There is an indication of the high-grade cell type as a consequence of viral etiology (10). The recent veterinary literature on tumors of domestic animals follows the Revised European-American Classification of Lymphoid Neoplasms (REAL), provided by the World Health Organization, which identifies each subtype of lymphomas as specific disease entities based on their cellular morphology, cell linage, and lesion topography (8). The application of this classification scheme in some studies indicated that the majority of EBL are diffuse large B-cell lymphoma (4,5,9).

The presumptive diagnosis of EBL is made by clinical and pathological findings. Subsequent confirmation depends on the BLV infection identification using numerous available serologic and/or molecular tests (eg, agar gel immunodiffusion enzyme-linked immunosorbent assay, radioimmunoassay, radioimmunoprecipitation assay, and polymerase chain reaction) (2). As only a percentage of infected cattle develop the disease, the presence of blood antibodies does not necessarily indicate the occurrence of clinical disease (6). These tests can also allow a range of clinical differential diagnoses. Enlargement of peripheral lymph nodes and progressive weight loss may be confused with tuberculosis, which can be differentiated by the tuberculin test (2). Traumatic pericarditis, chronic poisoning by cardiotoxic plants or ionophore, and bacterial valvular endocarditis should be considered in cattle with congestive heart failure (6). For the nervous clinical signs, the main diseases that should be excluded are spinal cord abscess, rabies, abscedative spondylitis, and medullary traumatism (2,6).

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