# Bovine Lymphosarcoma

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## INTRODUCTION

Bovine lymphosarcoma, also known as enzootic bovine lymphoma, is a malignant neoplastic disease of the lymphoreticular system in cattle that has a worldwide distribution.[11] It is the most common neoplasia of dairy cattle caused by bovine leukemia virus (BLV).[16] Prevalence of bovine lymphosarcoma is about 18 /100,000 cows slaughtered in the United States and it accounts for 27% of dairy condemnations at the slaughter house.[6, 14] Only one to five percent of cattle infected with bovine leukemia virus will actually develop lymphosarcoma.[11] Although dairy cattle are more commonly affected, bovine lymphosarcoma can effect beef cattle as well.[15] The natural hosts of BLV are water buffalo and cattle, but lymphoma can also develop in sheep.[1] BLV prevalence is usually higher in high milk producing cows since they stay in the milking herd longer.[12] There have been reports of BLV negative lymphoid tumors but these are rare.[16]

An important contributing factor in the development of lymphoma is the age of cattle.[15] Most bovine lymphosarcoma is diagnosed in cattle older than three years.[15] Bovine lymphoma can be divided into three disease manifestations: sporadic bovine lymphoma, enzootic bovine lymphosarcoma, and persistent lymphocytosis.[13, 7] In immature cattle, bovine lymphosarcoma is not usually associated with the bovine leukemia virus and can manifest as either juvenile, thymic or cutaneous forms.[9, 7] Juvenile sporadic lymphoma usually affects cattle less than 6 months old and causes enlargement of the peripheral lymph nodes.[7] Thymic sporadic lymphoma occurs in cattle less than two years of age and leads to rapid swelling in the brisket region.[7] Thymic lymphoma can led to difficulty eating and drinking which contributes to a poor prognosis.[7] Cutaneous sporadic lymphoma affects cattle from one to three years of age and cause nodes and plaques in skin.[7]

Adult cattle can develop either an enzootic bovine lymphosarcoma or a persistent lymphocytosis. [9, 7] Bovine enzootic lymphoma can lead to the development of bovine lymphosarcoma characterized by chronic low grade viremia with a long latency period and high fatality rate. The reservoir for bovine lymphosarcoma is persistently infected cattle.[11] The most commonly affected sites are the lymph nodes, abomasum, uterus, heart, and the spinal tract. Other sites of tumors that are less common include the kidney, ureters, retrobulbar space, mediastinal lymph nodes, and mesenteric lymph nodes. Persistent lymphocytosis is a benign elevation of the lymphocyte population. Cattle with persistent lymphocytosis will not show any clinical signs but will have an elevated white blood cell count.[7] Persistent lymphocytosis can predispose cattle to lymphosarcoma but not all cattle with persistent lymphocytosis will develop lymphosarcoma.

## HISTORY AND PRESENTATION

Pug, a five year old Simmental/Angus crossbreed, presented to MSU-CVM on 10/26/15 with a three week history of decreased appetite and body condition. Three weeks prior to presentation, the owner noted decreased body condition score and milk production. Additionally, Pug had a seven week old calf that stopped gaining weight 3 weeks previous to Pug presenting to MSU-CVM. The owner suspected the weight loss was secondary to parasitic infestation and treated Pug with fenbendazole (5 mg/kg PO). The referring veterinarian examined Pug and noted pyrexia (103.9°F), but no other significant physical exam findings were found. Fecal examination performed by the referring veterinarian revealed coccidia. At this time, Pug's referring veterinarian treated her with florfenicol (40 mg/kg IM), Vitamin B12 (2000 micrograms/cow PO), multivitamin, oxytetracycline (20 mg/kg IM), sulfamethazine (1 bolus/200 kgs body weight PO), flunixin meglumine (2.2 mg/kg SQ) and probios (15 grams PO). No

improvement occurred. On 10/25/15, the owner noted severe anorexia. Referring veterinarian physical examination revealed ataxia and bruxism. The referring veterinarian performed a CBC which revealed a leukocyte count of 78,000 wbc/microliter. Pug was referred to MSU-CVM Food Animal Department.

On presentation, Pug was depressed but responsive. She was underweight with a body condition score of three out of nine. On examination, Pug was open mouth breathing and exhibiting bruxism. Her capillary refill time was prolonged with a CRT of 3 seconds, and her vulvar lips were pale. Her respiratory rate, heart rate and temperature were elevated (Respiratory rate= 80 breathes per minute, Heart rate = 96 beats per min, Temperature= 104.2F). Jugular pulses were present and auscultation of her heart revealed a systolic murmur with a point of maximum intensity at the left heart base. Auscultation of her lungs was within normal limits. A wither's pinch test was performed and a decreased response was noted. An ultrasound of Pug's thorax and abdomen were performed which revealed fluid and fibrin in the pleural cavity and thickened abomasal walls and pyloris. Rectal examination revealed dark loose stool and multiple nodules in the uterus which is consistent with lymphosarcoma. A thoracocentesis was performed and yellow tinged fluid was collected and submitted for cytology. At this time a presumptive diagnosis of lymphosarcoma was made based on the clinical findings. Clinical findings in our case that are consistent with bovine lymphosarcoma include: anorexia, weight loss, heart murmur, thickened abomasal walls, and nodules in the uterus. An occult blood test was performed as a screening test for gastrointestinal bleeding. A presumptive diagnosis of Type II abomasal ulcers were also made due to Pug exhibiting bruxism, prolonged CRT, tachycardia, melena, and pale vulvular lips. Abomasal ulcers are common in cattle affected by bovine lymphosarcoma.

PATHOPHYSIOLOGY

The causative agent of enzootic bovine lymphoma is Bovine leukemia virus which is an oncogenic, B-lymphocytotropic, deltaretrovirus[2] The bovine leukemia virus causes infection by spreading through infected lymphocytes.[7] Transmission of BLV is accomplished by horizontal routes (direct routes, milk, insect bites, dehorning, ear tattooing and reused needles).[1] Once cattle are infected with BLV, they develop a subclinical infection. There are three manifestations of infection with BLV: clinically silent aleukemic state, persistent lymphocytosis (a benign polyclonal proliferation of B cells), and lethal lymphosarcoma.[2]

Once the virus has entered a new host it buds off and enters a new lymphocyte.[7] Once inside the new lymphocyte, the envelope and capsid disappear and the RNA strand copies itself and produces a new DNA strand.[7] The new DNA strand then inserts into the host DNA by reverse transcriptase enzyme.[7, 6] If cattle are genetically resistant they will have no viremia and will be seronegative.[7] Resistance and susceptibility to persistent lymphocytosis is associated with MHC Class II BoLA-DRB3 gene.[2] Studies have shown that peripheral mononuclear blood cells from aleukemic animals were found to express less IL-4 and IFN gamma.[8] The vast majority of cattle infected with bovine leukemia virus will be clinically normal, but will be seropositive and viremic.[7] Infection with BLV affects both the cells of the innate and adaptive immune systems.[8] BLV infection alters innate immune response by affecting the levels and production of cytokines.[8] BLV also leads to alteration of the humoral immune response by causing a polyclonal B cell expanision.[8] In infected cattle, 28-40% of the population will develop a persistent lymphocytosis and will also be viremic and seropositive.[7] Persistent lymphocytosis is a polyclonal expansion of B lymphocytes that mainly affects CD5+IgM+ B cells although it can affect CD4+ T cells, CD8+ T cells and gamma/delta T cells.[1, 7]

Persistent lymphocytosis cattle's peripheral blood mononuclear cells express less IL-2, IL-4, and IFN gamma mRNA than uninfected cattle.[8] The development of a persistent lymphocytosis predisposes cattle to the development of lymphosarcoma.[12] Although persistent lymphocytosis can predispose to lymphosarcoma, not all cattle with persistent lymphocytosis will develop clinical lymphosarcoma

Of all cattle that are seropositive, one to five percent will develop lymphosarcoma.[7] Infection of the cattle with BLV alone is not enough to lead to the development of lymphosarcoma so mutations such as a mutation in the p53 suppressor gene must also occur.[1] BLV encodes 2 regulatory proteins, Tax and Rex, in the pX region.[1] The main contributor to the oncogenic potential of BLV is the Tax protein[1]. BLV is suspected to cause leukomogenesis via the Tax protein. This protein functions as a transcriptional activator and causes immortalization of primary REFs.[1] The most common form of lymphosarcoma in adult cattle is multicentric lymphosarcoma, which affects the peripheral lymph nodes, liver, spleen, kidneys, heart and bowel.[5] Lymphosarcoma that affects the heart is localized to the right atrium or left ventricular myocardium.[6] Clinical signs associated with bovine lymphosarcoma include loss of condition, drop in production, anorexia, diarrhea, ataxia, and paresis.[6]

## DIAGNOSTIC APPROACH AND CONSIDERATION

Diagnosis of bovine lymphosarcoma is based on animal's age, clinical signs, antemortem tests, and necropsy findings.[6] Most cattle affected by lymphosarcoma will be between four to eight years of age.[7] Clinical signs with bovine lymphosarcoma are associated with the affected sites.[15] The most common presenting clinical signs in one study were anorexia, weight loss and

fever which is consistent with the presenting clinical signs in our case.[3] In our case clinical signs included decreased appetite, loss of body condition, decreased milk production, elevated temperature, bruxism, heart murmur, prolonged CRT, pale mucous membranes, jugular pulses, ataxia and uterine masses on rectal palpation. Antemortem diagnostic tests for bovine lymphosarcoma include peripheral lymph node wedge biopsies, surgical exploration, biopsy, pleurocentesis, pericardiocentesis, peripheral lymph node fine needle aspirate, abdominocentesis, serological tests and cerebral spinal fluid tap.[3] The antemortem tests performed in our case were CBC, Chemistry, thoracocentesis, occult blood test, thoracic ultrasound and abdominal ultrasound. In Pug's case, bloodwork revealed leukocytosis and anemia. Anemia in this case is likely associated with Type II abomasal ulcers which are very common in cattle with lymphosarcoma due to the fact Pug was exhibiting bruxism, melena, prolonged CRT, tachycardia, and pale vulvular lips. Pug had a severely elevated lymphocyte count at 60,700/ microliter which is consistent with a persistent lymphocytosis. Thoracic ultrasound revealed fibrinous material and pleural fluid. Abdominal ultrasound revealed a thickened abomasal wall and pyloris, and dilation of the portal triad. Thoracentesis revealed cloudy yellow tinged fluid which was evaluated and determined to have a mixed population of cells that consisted of neutrophils, macrophages, small lymphocytes, numerous immature lymphocytes and lymphoblasts, and several mitotic figures consistent with lymphosarcoma. The combinations of Pug's clinical findings lead to the confident diagnosis of lymphosarcoma. Due to her poor prognosis and lack of treatment options, Pug was humanely euthanized. Confirmation of the diagnosis of lymphosarcoma requires a necropsy with biopsies of the lesion so a necropsy was performed.

## TREATMENT AND MANAGEMENT

There is no cure for bovine lymphosarcoma and treatment is limited to corticosteroids, chemotherapy agents, and supportive therapy only in genetically valuable animals.[6] Typically, treatment is only indicated when trying to keep an animal comfortable or to prolong life to harvest calf, ova, or semen.

Control of BLV infection is difficult due financial constraints and the difficulty of implementing a control program.[6] Western European countries, Australia, and New Zealand have developed eradication programs, but there is little incentive in the United States to implement control programs.[10, 17, 7] Persistent lymphocytosis cattle can act as a reservoir for spread of BLV by spreading infection horizontally and transplacentally.[10] Detection of BLV in milk has been used to determine how prevalent BLV is on dairy farms and can aid in the control of spread by detecting reservoirs.[17] Some other control options for BLV are test and slaughter, segregation of cattle and management changes.[4] Horizontal transmission is the main point of transmission that needs to be controlled.[4] Some ways to control horizontal transmission are: cleaning the maternity pen and removing the calf soon after birth, feed colostrum from negative cows, freeze colostrum, separate positive and negative cows, use needles once, clean and disinfect equipment between animals, change obstetrical sleeves between cows, artificial insemination, and vector control.[4] Testing for positive animals can be used to determine which animals have been infected and to monitor for increase in the number of infected animals which can suggest increased horizontal transmission.[4] Recipients for embryo transfer should also be negative to minimize the spread of bovine leukemia virus.[6]

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CASE OUTCOME

Pug was euthanized using captive bolt followed by KCL administration. After euthanasia, a necropsy was performed, which revealed lesions consistent with lymphosarcoma. External examination during necropsy revealed a body condition score of three out of nine and slightly bulging eyeballs. There was approximately 100 mls of yellow tinged fluid in the abdominal cavity and serosanguinous fluid in the thoracic cavity. Focal fibrinous adhesions were noted that connected the right lung to the rib cage. The liver was moderately enlarged with rounded edges. The peripheral lymph nodes were noted to be enlarged. The cut surface of the lymph nodes revealed multifocal white nodules nodules that were approximately 2 X 2 cm in size. The abomasum was distended and firm and on cut surface the abomasal wall was 2-3X's the normal thickness. There was a 30-40 cm mass extending from the abomasal wall and encompassing the omasum. Thick fibrinous to fibrous material was present on the epicardial surface. The walls of all the chambers of the heart were thickened and mottled pale cream to pale red. The entire left horn of the uterus contained ½-2 cm multifocal raised nodules that had smooth surfaces. The left horn also had a moderate amount of green tinged gritty fluid present. In the cervix, there was a 2 cm X 3cm raised irregular white to cream mass that extended into the lumen. Necropsy findings led to the definitive diagnosis of lymphosarcoma of the heart, abomasum, uterus, liver, spleen and lymph nodes. Lesions on the heart also lead to the diagnosis of fibrinous epicarditis. Histopathology revealed neoplastic lymphocytes in the abomasum, mass around the omasum, heart, uterus and lymph nodes which confirmed diagnosis of lymphosarcoma.

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