

“Big Belly, Big Brisket, Little Bovine”

Elizabeth A. Orr

Mississippi State University

College of Veterinary Medicine

Class of 2021

Clinicopathological Conference

September 18, 2020

Advisor:

Gretchen Grissett, DVM, MS, DACVIM-LA

Assistant Clinical Professor

Introduction

Cattle are more frequently diagnosed with neoplasia than other food animal species.

Lymphosarcoma is a common neoplasm in cattle, with an estimated 50% of dairy cattle condemnations at slaughter resulting from lymphosarcoma. The term bovine leukosis is a generalized term encompassing both enzootic bovine leukosis (EBL) and sporadic bovine leukosis (SBL). Cattle with EBL are infected with the Bovine Leukosis Virus, whereas SBL is considered a rare disease (0.001%) with no known etiology. Although EBL is considered common, approximately 5% of cattle infected ever develop lymphosarcoma. Enzootic bovine leukosis is endemic in most countries, outside of the EU, where it has been eliminated through extreme measures of testing and culling. The greatest prevalence in the US is in the southeastern region of the country.^{1,7,11}

History and Presentation

G015, an 11-month old beef cross heifer, presented to MSU-CVM Food Animal Department on 3/2/20 for bloat. She was observed isolating herself from the herd approximately 2 weeks ago prior to presentation. Roughly one week prior to presentation, G015 had a hard "swelling" around her jaw and brisket. At that time, she was brought up from pasture and given fenbendazole orally and an ivermectin injection for presumed gastrointestinal parasitism. The morning of 3/2/20, G015 was found severely bloated and was immediately brought to MSU. G015 had access to pasture, a high Mg mineral, and a protein tub. No other animals on the premises appeared affected.

Upon presentation, G015 was quiet, alert, and responsive. She weighed 206 kgs with a body

condition score of 3/9. She had a rectal temperature of 103.2, heart rate of 72, and respiratory rate of 36. Her heart and lungs were normal on auscultation. She had pale pink mucous membranes and a CRT of <2 seconds. Normal gut sounds and 1 rumen contraction per minute were appreciated. She was severely distended on her left side (bloat). A firm mass extended the length of her neck from just cranial to her larynx down to her brisket and just cranial to her right shoulder. Submandibular edema was also visible. Both mandibular and prescapular lymph nodes were severely enlarged, and her right femoral was slightly enlarged. Her jugular veins were distended with jugular pulses present.

Pathophysiology

Sporadic bovine leukosis has no known etiology and is divided into the following forms: cutaneous, multicentric, and thymic.

The cutaneous form is discussed the least in literature. It is often found in cattle 1-3 years of age and is characterized by superficial skin lesions commonly seen on the perineum, udder, dorsum, and pelvic limbs. The lesions can vary in appearance from thickened, ulcerated plaques to more firm rounded nodules. Lymphadenopathy may or may not be present. Though rare, this form has been reported to both regress completely as well as progress into a more generalized, multicentric lymphosarcoma.^{7,11}

Multicentric, calf, and juvenile are all terms used to describe the form of SBL that most frequently affects calves 1-6 months of age. This form is characterized by a generalized lymphadenopathy with involvement of multiple organ systems. However, regional lymphadenopathy without infiltration to internal organs has been reported in this form as well. Calves with multicentric SBL tend to show more systemic signs of illness, such as lethargy,

weight loss, fever, anorexia, and anemia. Other clinical signs are based on the organs affected (spleen, thymus, heart, liver, kidneys, and spine). Both multicentric and thymic SBL are often fatal within one to two months of exhibiting clinical signs. ^{1,7,10,11}

The other rapidly fatal form of SBL is the thymic or adolescent form, which is commonly seen in cattle 6-24 months of age. Like G015, patients often present for recurrent bloat and a solid mass in the cervical region. Signs are often related to the extensive size of the tumor/thymic tissue. As it compresses the lymphatic system and jugular veins, facial and cervical edema as well as prominent, distended jugulars become visible. Inadequate ventilation and bloat are common sequelae to the compression of the trachea and esophagus. Pulmonary effusion, lethargy, weight loss, and regional lymphadenopathy are also frequently seen. Opposite of EBL, thymic SBL is more common in beef than dairy, with some evidence suggesting a genetic component. The most significant study supporting this theory looked at 216 cases of thymic lymphoma in France in which majority of the calves were sired by the same bull. ^{1,7,9,10,11} There are multiple case reports that discuss calves with SBL that cannot be categorized into any of the three forms, but instead take on characteristics of 2 or more and are labeled atypical SBL. Though unlikely, it is important to be aware of this conundrum. ¹¹

Unlike sporadic bovine leukosis, enzootic bovine leukosis has a known etiologic agent – the bovine leukemia virus (BLV). Bovine leukemia virus is an oncogenic retro virus (related to the human t-lymphotropic virus type 1 (HTLV-1)) that infects lymphocytes. The virus has reportedly been detected in T cells. However, the disease, both lymphocytosis and lymphosarcoma, is considered B-cell in origin, whereas SBL is more frequently T-Cell in origin. ⁶ As with HTLV-1, BLV strategically evades the immune system by replicating at a minimal rate and keeping B-cells from surfacing viral proteins. Although an infected animal remains infected for life, like

HTLV-1, BLV rarely causes clinical disease. Approximately 30% of infected animals will exhibit lymphocytosis and 5% will succumb to lymphosarcoma. Most with chronic lymphocytosis never show any clinical signs. Decreased milk production, immunosuppression, as well as a greater probability of infecting their calf in utero have been reported as sequelae to chronic lymphocytosis. Other than lymphadenopathy, clinical signs of lymphosarcoma relate to the location of the lesions. Common sites affected include the heart (arrhythmias, murmurs, failure), abomasum (GI signs-ulcers, melena), uterus (failure to conceive, abortion), spine (pelvic limb paresis or paralysis), and retrobulbar area (exophthalmos to proptosis). Lymphosarcoma is most frequently seen in cattle 3-8 years of age. ^{1,2,7,11}

Though biting flies and other insects were originally thought to be the primary route of transmission, research suggests that iatrogenic spread of the virus through certain management practices is more probable. Any animate or inanimate object with infected blood on it can act as a vector, such as needles, dehorers, tattoo pliers, etc. Though unlikely, it is possible for BLV to be transmitted sexually (via live cover). Current literature suggests that BLV can also be spread in-utero (5%) and through milk (unlikely via colostrum). Bovine leucosis virus is more common in dairy herds than beef, which is attributed to the different management practices. ^{1,2,3,7,11}

Diagnostics

Before pursuing diagnostics, an orogastric tube was obtained to relieve G015's bloat. However, the tube could not be placed due to an obstruction. A presumptive diagnosis of sporadic bovine lymphosarcoma was reached based on signalment, history, clinical signs, and physical exam. Blood was collected for educational purposes. The CBC and Chemistry were both unremarkable. CBC revealed a mild neutrophilia and mild lymphopenia. Chemistry revealed a mild increase in AST, globulins, and total bilirubin, and a mild decrease in albumin, calcium, and magnesium.

As in G015's case, both BLV and SBL are routinely diagnosed by history and PE alone. Further diagnostics that can be utilized include - CBC, FNA and cytology, biopsy, and testing for the presence of BLV (AGID, ELISA, PCR). Animals diagnosed with SBL can also test positive for BLV; this is a coincidental finding and unrelated to their disease. In cases of BLV-associated lymphosarcoma, a CBC often reveals marked lymphocytosis. CBC and Chemistry will likely be unremarkable in both thymic and cutaneous SBL. CBC in calves with multicentric SBL may reveal lymphocytosis with or without anemia (microcytic, hypochromic) and thrombocytopenia due to bone marrow necrosis. Cytology from a fine needle aspirate is often heavily contaminated with blood and difficult to assess. However, a monomorphic population of lymphocytes with varying mitotic figures is diagnostic in all forms of the disease. There will be slight differences in size, appearance, and mitotic rates between the different forms, specifically between EBL and SBL.^{1,7,11}

With regards to testing for BLV, the presence of BLV does not mean the animal is clinically ill or will ever exhibit clinical disease. Further research is needed, however there is evidence suggesting that cattle with the bovine MHC class II DRA/DRB3*0902 allele have significantly lower proviral loads (meaning less likely to develop lymphosarcoma) than those with other DRB3 allele variants. This, and other research looking at specific viral protein (gp51) mutations that enhance disease progression, suggests a potential for diagnostic tests with the ability to identify which animals are likely to develop clinical disease.¹²

Treatment:

Unfortunately for G015, the treatment for SBL is humane euthanasia. As mentioned before, there is a small chance that the cutaneous form will regress; however, the multicentric and thymic forms are always fatal. With an unknown etiology, hope for future treatment options is abysmal.

In cases of EBL, many hope a safe and effective vaccine becomes commercially available. Numerous attempts have failed in the past due to the virus's ability to evade the immune response.⁵ Prevention is currently the only treatment option available for EBL. Testing and culling or even testing and segregating is only feasible in herds with an extremely low prevalence. In herds with a high prevalence of BLV positive animals, altering management practices to minimize spread is suggested. These practices include: changing out needles between each animal, cleaning/disinfecting tools and instruments between use, separating BLV-positive cows from calves, feeding calves milk from BLV-negative dams, changing OB sleeves between cows, utilizing artificial insemination, and controlling fly populations.⁸

Case Outcome:

After being humanely euthanized, G015's body was necropsied. The presumptive diagnosis of thymic sporadic lymphoma was confirmed on gross and histologic examination. On gross examination, a multi-lobulated, white, firm, dense mass (19cm x19cm x5.5cm) was observed extending from the thoracic inlet to 15cm caudal to the larynx. The trachea was compressed and contained a frothy fluid throughout. The esophagus was displaced dorsally, and a bloat line was visible. Multiple lung lobes were wet and heavy, with coalescing dark red areas present. Both prescapular and mediastinal lymph nodes were markedly enlarged and bulged on cut surface. Several round, white masses were observed cranial to the heart, although the heart did not exhibit any abnormalities. The rumen was severely distended due to gas, and there were several severely enlarged abdominal lymph nodes. No other abnormalities were observed on gross examination. Histologic examination of the cervical mass revealed small round cells with scant eosinophilic cytoplasm and round nuclei. The nuclei had finely stippled chromatin and indistinct nucleoli.

The mitotic rate was low at 0-1 per high powered field. One study suggests that tumors due to EBL exhibit much higher mitotic rates than those from SBL. ⁴

Conclusion

In conclusion, lymphosarcoma is one of the most frequently reported neoplasms in cattle. Lymphosarcoma in cattle is the clinical manifestation of either enzootic bovine leukosis or sporadic bovine leukosis. Sporadic bovine leukosis is further subdivided into three forms: cutaneous, multicentric, or thymic, with multicentric and thymic being 100% fatal. Sporadic bovine leukosis has no known etiologic agent, diagnosis is most frequently based on history and physical exam (+/- cytology/biopsy), and treatment is humane euthanasia. Enzootic bovine leukosis is considered endemic in most parts of the world and affects adult cattle. The agent is bovine leukemia virus, which remains in the animal lifelong once infected. The virus easily evades the immune system and rarely causes clinical disease. Approximately 5% of all cattle infected will develop lymphosarcoma. AGID, ELISA, and PCR tests are all available to detect the presence of the virus in an animal. Treatment for lymphosarcoma in BLV-infected cattle is also euthanasia, and no vaccine is currently available. Since transmission is most likely iatrogenic, tailoring management practices to mitigate the spread is the greatest tool available to minimize the presence of this disease. ^{1,7,11}

References:

1. Nagy, Dusty W., et al. "Overview of Bovine Leukosis - Generalized Conditions." *Merck Veterinary Manual*, Merck Veterinary Manual
2. "Bovine Leukosis Virus." *Cornell University College of Veterinary Medicine*, 15 May 2019, www.vet.cornell.edu/animal-health-diagnostic-center/programs/nyschap/modules-documents/bovine-leukosis-virus
3. Watanuki, S., Takeshima, S., Borjigin, L. *et al.* "Visualizing bovine leukemia virus (BLV)-infected cells and measuring BLV proviral loads in the milk of BLV seropositive dams." *Vet Res* 50, 102 (2019), doi:10.1186/s13567-019-0724-1
4. Vernau, W., et al. "Classification of 1,198 Cases of Bovine Lymphoma Using the National Cancer Institute Working Formulation for Human Non-Hodgkin's Lymphomas." *Veterinary Pathology*, vol. 29, no. 3, May 1992, pp. 183–195, doi:10.1177/030098589202900301.
5. Abdala, Alejandro et al. "BLV: lessons on vaccine development." *Retrovirology* vol. 16,1 26. 7 Oct. 2019, doi:10.1186/s12977-019-0488-8
6. YIN, Shan-ai, et al. "Relation between Phenotype of Tumor Cells and Clinicopathology in Bovine Leukosis." *Journal of Veterinary Medical Science*, JAPANESE SOCIETY OF VETERINARY SCIENCE, 6 June 2003, www.jstage.jst.go.jp/article/jvms/65/5/65_5_599/_article
7. Nasir, Karen S. "Sporadic juvenile thymic lymphoma in a 6-month-old Holstein heifer." *The Canadian Veterinary Journal*, vol. 46,9 (2005): 831-3.
8. "Options for Controlling BLV." *BLV*, *Michigan State University*, <https://blv.msu.edu/control/index.html>

9. Da Costa, B et al. "Epidemiological and pathological studies of a familial thymic lymphosarcoma in bovine species." *Leukemia* vol. 5,5 (1991): 420-4.
10. Bezerra, et al. "Sporadic multicentric lymphoma in a Nelore calf." *Brazilian Journal of Veterinary Pathology*, 2015, 8(2), 65 – 67, <http://bjvp.org.br/wp-content/uploads/2015/07/v8-n2-5.pdf>
11. Hendrick, Steven H. "Atypical sporadic bovine leukosis in a beef feedlot heifer." *The Canadian Veterinary Journal*, vol. 43,8 (2002): 617-9.
12. Lo, C.-W.; Borjigin, L.; Saito, S.; Fukunaga, K.; Saitou, E.; Okazaki, K.; Mizutani, T.; Wada, S.; Takeshima, S.-N.; Aida, Y. "BoLA-DRB3 Polymorphism is Associated with Differential Susceptibility to Bovine Leukemia Virus-Induced Lymphoma and Proviral Load." *Viruses* 2020, 12, 352