6th Annual All Things BLV Meeting

MICHIGAN STATE UNIVERSITY

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October 22, 2021

Woldumar Nature Center
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Welcome to the 2021 All Things BLV Meeting at Michigan State University!

This meeting provides an overview of the research, collaborations, and knowledge gained from the team and will provide an update on All Things Bovine Leukemia Virus at Michigan State University. We are pleased that you could join us, and we look forward to all future collaborations and advancements we will gain as a result of hosting this meeting.

BLV Research Team
MICHIGAN STATE UNIVERSITY

Any questions or meeting needs can be sent to:

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Application of Novel Diagnostics and Phylodynamics to Address Control of Bovine Leukemia Virus (BLV) in Dairy Cattle

Scott Wells[*], Nirmala Jayaveeramuthu, Vikash Singh, Tatiana Lenskaia, Sunil Kumar Mor, and Declan Schroeder

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Control of Bovine Leukemia Virus (BLV) in US dairy herds is challenging due to very high infection prevalence and multiple blood and milk-borne pathways of transmission. Effective vaccination or treatment is not available, and BLV control depends on management to prevent transmission to susceptible cattle. Methods to identify specific transmission pathways for individual farms are not yet available, which could help to prioritize disease control measures. The objective of this study was to identify primary routes of BLV transmission within study herds through linking BLV variant and epidemiologic data using phylodynamics methods. From two BLV-infected study herds (approximately 140 and 170 cattle), blood samples were collected from cattle 6 months of age or older at 3 samplings at 6 month intervals and submitted to the Minnesota Veterinary Diagnostic Laboratory for testing using a PCR assay (CentralStar Cooperative, East Lansing, MI) to detect BLV DNA. Following DNA extraction from PCR-positive cattle, targeted PCR based near complete whole genome sequencing using the Oxford Nanopore Technologies (ONT) GridION platform was performed. Phylogenetic inference trees were generated based on consensus genomes obtained for each positive cow and cow-level epidemiologic data was used to identity trends to confirm known possible transmission routes. PCR test results identified over 29% of cattle were test-positive at each sampling in the 2 study herds. In one herd, the incidence of new test-positive cattle was higher following the summer season (27%) compared to the winter season (3%). In the other herd, a similar incidence of new test-positive cattle was detected across seasons (11-12%). From evaluation of several cattle that tested positive at multiple sample dates, similar BLV variant co-infections were identified within cattle (82% in one herd and 58% in the other), which also indicates the potential existence of mixed BLV infections within some cattle. From evaluation of BLV-test positive dam-daughter pairs of cattle identified, identical or bootstrap supported identities of BLV variants were identified within dam-daughter pairs (25% and 33% in each herd, respectively). This data provides a preliminary estimate of the contribution of dam-daughter transmission to BLV infection in these study herds. Evaluation is underway to gain further understanding of BLV transmission within dairy cattle herds both in space and time.

Tracing Viral Transmission and Evolution of Bovine Leukemia Virus Through Long Read Oxford Nanopore Sequencing of the Proviral Genome

Laura A. Pavliscak[1*], Jayaveeramuthu Nirmala[2,3], Vikash K. Singh[2], Kelly R. B. Sporer[1], Tasia M. Taxis[4], Pawan Kumar[3], Sagar M. Goyal[2], Sunil Kumar Mor[2,3], Declan C. Schroeder[3,5], Scott J. Wells[3], and Casey J. Droscha[1,4]

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Bovine leukemia virus (BLV) causes Enzootic Bovine Leukosis (EBL), a persistent life-long disease resulting in immune dysfunction and shortened lifespan in infected cattle, severely impacting the profitability of the US dairy industry. Our group has found that 94% of dairy farms in the United States are infected with BLV with an average in-herd prevalence of 46%. This is partly due to the lack of clinical presentation during the early stages of primary infection and the elusive nature of BLV transmission. This study sought to validate a near-complete genomic sequencing approach for reliability and accuracy before determining its efficacy in characterizing the sequence identity of BLV proviral genomes collected from a pilot study made up of 14 animals from one commercial dairy herd. These BLV-infected animals were comprised of seven adult dam/daughter pairs that tested positive by ELISA and qPCR. The results demonstrate sequence identity or divergence of the BLV genome from the same samples tested in two independent laboratories, suggesting both vertical and horizontal transmission in this dairy herd. This study supports the use of Oxford Nanopore sequencing for the identification of viral SNPs that can be used for retrospective genetic contact tracing of BLV transmission.

Phenotypic Selection of Dairy Cattle Infected with Bovine Leukemia Virus Demonstrates Immunogenetic Resilience Through NGS-based Genotyping of BoLA-DRB3 MHC Class II Genes

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Characterization of the bovine leukocyte antigen (BoLA) DRB3 gene has shown specific alleles associate with susceptibility or resilience to the progression of bovine leukemia virus (BLV), measured by proviral load (PVL). Through surveillance of multi-farm BLV eradication field trials, we observed differential phenotypes within seropositive animals that persist from months to years. We sought to develop a multiplex next-generation sequencing workflow (NGS-SBT) capable of genotyping 384 animals per run to assess the relationship between BLV phenotype and two BoLA genes. To phenotype animals, we utilized longitudinal results from milk ELISA screening and subsequent blood draw on seropositive animals for PVL determination using a novel BLV Proviral Load multiplex qPCR assay. Repeated diagnostic observations defined two distinct phenotypes in our study population, ELISA-positive animals that do not harbor detectable levels of provirus and those who do have persistent proviral loads. In total, 558 cows- 168 BLV susceptible (ELISA-positive/PVL-positive) and 390 BLV resilient (ELISA-positive/PVL-negative) from 9 Midwest dairy farms were selected for NGS-SBT. Three BoLA-DRB3 alleles, including one novel allele, were shown to associate with disease resilience, *009:02, *044:01, and *048:02 were found at rates of 97.5%, 86.5%, and 90.3%, respectively, within the phenotypically resilient population. Alternatively, DRB3*015:01 and *027:03, both known to associate with disease progression, were found at rates of 81.1% and 92.3%, respectively, within the susceptible population. This study helps solidify the immunogenetic relationship between BoLA-DRB3 alleles and BLV of these two phenotypic groupings of US dairy cattle.

Calf Expressed MicroRNAs Associated with Dams Infected with Bovine Leukemia Virus

Ciarra LaHuis[1*], Turner Swartz[1], Kelly R.B. Sporer[2], Casey Droscha[2], and Tasia M. Taxis[1]

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The 2007 USDA survey estimated 89% of dairy operations in the United States were seropositive for Bovine Leukemia Virus (BLV). Bovine leukosis is the disease caused by BLV and leads to increased susceptibility to opportunistic infections. Cattle infected with BLV often suffer impaired immune function. Further, about 5% of infected cattle will develop lymphomas. MicroRNAs are a small, noncoding RNA species that are involved in post-transcriptional gene regulation. Research that explores the associations between BLV and host microRNA profiles is lacking. While research provides evidence that microRNAs influence immune development in calves, no research has addressed the potential influence of maternal BLV infection status on progeny microRNA expression. Our project aims to identify calf microRNAs influenced by a dam's BLV status. Blood samples from dam and calf pairs were collected from animals at the Dairy Cattle Teaching and Research Center at Michigan State University. Dams were sampled 14 to 30 days prior to parturition, and again within 24 hours of parturition (n= 51). Antibodies for BLV were assessed via ELISA, as well as the amount of provirus or proviral load (PVL) via qPCR for all dams. Calves were sampled on days 0, 7, 14, and 21 of life (n=51). White blood cells (WBCs) were extracted from all calf samples and dams within 24 hours of parturition. In further analysis, RNA will be extracted from WBC samples to sequence microRNAs. Counts of calf microRNA sequences will be assessed to determine associations between dam BLV infection status and the calf. We hope to identify expression of microRNAs in calves born to both ELISA positive and ELISA negative dams and account for the varying levels of PVL in ELISA positive dams. We will continue to investigate the potential impact that dam BLV infection has calf development. It is imperative to look further into these potential associations as microRNAs play a role in gene function, and this may impact the development of calves on a dairy operation.

Managing Bovine Leukemia Virus by Integrating Surveillance of Young Stock and Whole Herd Scans

Madison E. Sokacz[1*], Paul Bartlett[2], Casey J. Droscha[3], Kelly R. B. Sporer[3], Bo Norby[2], and Tasia M. Taxis[1]

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Our research strives to improve the sustainability of the US dairy industry by developing and testing methods to reduce the prevalence of Bovine Leukemia Virus (BLV). It was previously believed that BLV primarily affected older cows, but field trial data indicated that first lactation cows were infected with BLV. Our study will aid in identifying when BLV infections occur among young stock while continuing to identify and manage infected cows in the milking herd. Dairy farms will be surveyed to learn calf and cow management protocols before enrollment into the study. Blood samples will be collected from female neonates (24 hours to 8 days in age), then, one year in age, first pregnancy check, and at 60 days in milk for each lactation. Following collection, blood samples will be tested for BLV antibodies (via ELISA) and provirus (via qPCR). This longitudinal profiling will provide evidence of BLV infection dynamics within infected herds. In tandem with profiling, the milking herd will be screened for BLV every other year. Milk samples will be collected and tested for BLV antibodies. Blood samples will then be collected from cows with BLV antibodies to quantify provirus. Summarized results will be conveyed to producers to use for mitigation strategies. Currently, five Michigan farms are enrolled in the study. Three farms milk >500 cows with a 38% (± 6.98) average BLV prevalence and two farms milk <500 cows with a 55.5% (± 0.77) average BLV prevalence. Seven months since the commencement of the project, 171 female neonates have been sampled and therefore enrolled in the project to be profiled throughout their life span. Of those, 33.33% have BLV antibodies and 1 calf has detectable provirus. The first round of screening the milking herds on all 5 farms is planned for completion by January 2022. Our integrated approach of tracking BLV incidence and managing cows already infected is novel and will give producers an integrated approach to managing BLV. This will work to improve animal welfare and work towards a more sustainable industry.

Changes in Bovine Leukemia Virus Diagnostic Parameters in Naturally Infected Dairy Cattle Over a Lactation Cycle

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Bovine leukemia virus (BLV) is a delta-retrovirus which infects the B lymphocytes of cattle. 46% of all U.S dairy cattle are estimated to be infected with BLV. Our objectives are to: 1) determine if common parameters used to diagnose BLV infection change over time and 2) identify important time points for new BLV infections over a lactation period in dairy cattle. Two cohorts of 44 animals each were enrolled at 150±4 days prior to calving and included animals about to enter their first or greater lactation. Blood samples were collected at enrollment, then every 2 weeks until calving, and then every 4 weeks until the next dry-off. BLV serum ELISA testing was performed at each collection time point. CBCs were run every ~4 weeks from enrollment to ~60 days in milk (DIM) and once at ~120 DIM. Mean lymphocyte counts (units of: $x10e3/uL$) at ~60 days prior to parturition were 6.43 for BLV+ and 3.54 for BLV- animals which was significantly different ($p<0.01$). However, mean lymphocyte counts fell for the BLV+ group and became non-significant from the BLV- group just after dry-off and preceding and following calving. Using a repeated measures linear mixed model, BLV status ($p<0.01$), time ($p<0.01$), and lactation of 3+ ($p<0.01$) all had significant effects on lymphocyte count. ELISA optical density (OD) values increased at dry-off and ~30 days post calving in all BLV+ animals. Five animals sero-converted over the first 8 months and initial qPCR in sero-converting and BLV+ animals showed proviral load (PVL, # viral copies/10^3 leukocytes) fluctuations over time. This study shows that lymphocyte count, BLV OD and BLV PVL change throughout a lactation cycle and may be caused by the impact of stress on viral reactivation and replication followed by immune system activation and clearance of some infected lymphocytes.

The Impact of Bovine Leukemia Virus on Dairy Longevity

Oscar J. Benitez[1], Rebecca M. LaDronka[2], Bo Norby[3],
Daniel L. Grooms[4], and Paul C. Bartlett[3*]

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A prospective study of 91 dairy herds in Michigan showed decreased cow longevity/lifespan in BLV antibody-positive cows as compared to their negative herdmates. Our objective was to determine the association between BLV infection and cow longevity in their herds. Ninety-one dairy herds from 9 U.S. states volunteered to participate in this study. Female dairy cattle (n=3,611) were tested for BLV antibodies with an ELISA milk test, and their presence in the herd was monitored for an average of 29 months. The survival analysis controlled for herd and lactation number. Cows sold for dairy purposes were excluded, and the individual-cow results were not shared with the producers so as to not influence culling decisions. Overall, 47.1% (1,701/3,611) of cows were ELISA-positive. The significant hazard ratio of 1.30 indicated that ELISA-positive cows were 30% more likely than their negative herdmates to die or be culled during the monitoring period. Reasons given by the producers for the cows being culled did not differ between BLV-positive and BLV-negative cows, however cows reportedly "found dead" was significantly more frequent among the BLV-positive cows. These results are consistent with other reports that BLV infection negatively impacts the wellbeing and productivity of dairy cattle.

The Economic Impact of Bovine Leukemia Virus in Michigan Dairies

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Department of Agricultural, Food, and Resource Economics, East Lansing, MI

Economic impacts of Bovine leukemia virus (BLV) on the dairy industry have previously been made. However, the previously proposed economic measures are limited due to a lack of farm level data, resulting in economic models and estimates with numerous assumptions about costs, disease prevalence, and producer decisions regarding BLV. Our research project will utilize farm and cow level financial and animal data from five Michigan dairy farms, allowing for a more accurate measure of the economic impact of BLV. Primary financial data including milk and cull prices, feed purchases, labor costs, and health care costs, will be collected through TelFarm, a farm financial record keeping system. Biological and production data will be collected from the farm's herd management software systems. BLV positive cows as well as anti-body levels on a per cow basis will be identified from data collected through a co-PI's concurrent study (USDA-AFRI Award #2020-67015-31562). Economic evaluations will use partial costs and net revenue equations, as well as regression and sensitivity analyses over 10 years. We will evaluate different test and cull strategies as well. Results from this study will estimate the profitability impacts of BLV on a per cow basis and simulate profitability impacts over a 10 year period. These estimates will include herd health and cattle longevity impact. Furthermore, an interactive partial budget decision tool will be created which allows for input of an individual's farm financial and biological data to determine potential profitability impacts for the farm based on different BLV management strategies. Outreach events and workshops will be held to disseminate our findings. Our research will allow Michigan dairy producers to more accurately assess the economic impact of BLV, with the ultimate goal to reduce BLV prevalence and improve profitability. This study is being funded by the Michigan Alliance for Animal Agriculture (M-AAA).

The Impact of Removal or Segregation of Cattle That Have a High Proviral Load of Bovine Leukemia Virus (BLV) in Reducing Incidence and Prevalence of BLV in Dairy Herds in the Canadian Maritimes

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The prevalence of BLV at the herd level is 89.3 % (John et al 2019) in Atlantic Canada and is increasing year after year despite most of the herds applying the standard measures to control horizontal transmission. Targeted culling or segregating the likely most infectious cow by measuring their proviral load (PVL) has been reported to decrease the prevalence in 2 pilot studies in the USA. The objective of this study is to evaluate the impact that culling and segregation of cows with high PVL has on reducing the prevalence and incidence of BLV in dairy herds in the Maritimes. Thirty dairy herds from the three Maritime provinces were enrolled in this study. True prevalence in each herd was calculated by individual BLV ELISA test in milk or blood for lactating, dry and bred heifers. For each BLV-positive cow, a qPCR test was performed to identify their PVL. This will be repeated every year for a 3-years period. The incidence and prevalence of BLV for each herd will be calculated every year. Cows with moderate to low PLV will have their PVL retested every year if they remain in the herd. Multivariate Cluster analysis was performed to identify any similarity of PVL level between herds. A total of 4051 cows were tested and 1,375 were BLV-positive. Within herd prevalence of BLV ranged from 19% to 89%. From the 1,375 BLV-positive cows, 473 were classified as high-shedders (PVL>1 viral copy/wbc). The percentage of the high, moderate, low, an undetected PVL were 29%, 10% ,30% and 31%, respectively. A total of three herd-clusters were identified: a small cluster with high proportion of high PVL cow/BLV positive cow (more than 50%), large cluster with a proportion of 10-15% and another small cluster with proportion less then 5%. About 1/3 of BLV positive cows have a high PVL. Focusing culling or segregation decisions on this group will likely be more economically feasible in reducing and controlling BLV in their herd.

Effect of Selective Removal of Cattle with High Proviral Load on the Herd-Level Prevalence of Bovine Leukosis

Sulav Shrestha[1*], Karin Orsel[1], Herman W. Barkema[1,2], Guido van Marle[2], M. Faizal Abdul-Careem[1], and Frank van der Meer[1]

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Bovine leukemia virus (BLV), as a retrovirus, produces a copy of itself and consolidates into the host's genome, thus forming a provirus. The number of proviruses in an infected animal differs which is why not all infected animals show clinical signs. The proviral load (PVL) is measured as the number of proviruses per host's white blood cell. Studies have indicated that high proviral load (HPL) animals have a higher likelihood of transmitting the virus. The objective of this study was, therefore, to assess the impact of removing the HPL cows from the herd on the herd-level BLV prevalence. Eleven free-stall dairy herds across Alberta with a herd size ranging from 81 to 402 cows were enrolled in a three-year study. In the first year (2020), 768 out of 2,301 milking cows were ELISA positive for BLV antibodies in milk or serum. Blood from the positive cows was further assessed to determine their proviral load using the BLV SS1 qPCR assay (CentralStar Cooperative Inc.). The within-herd BLV prevalence ranged from 9.2 to 52.2% (median = 33.9%). The proportion of HPL cows out of the total BLV-infected cows per herd ranged from 3.3 to 18.6% (Median = 10.5%). Compared to the total cows tested per herd, the proportion of HPL cows varied from 0.7 to 7.4% (Median = 3.7%). It was recommended to remove the HPL cows from the herd as removal of all BLV-infected animals is not feasible given the current prevalence. Currently, the herds are monitored to assess the impact of the removal strategy on the BLV prevalence as part of the second year activities which will be repeated in 2022.

Moving to Zero – A Pathway to Eradicate BLV From Dairy Herds

Phil T. Durst[1*], Tasia Kendrick[2], Kelly R. B. Sporer[3], Casey Droscha[3], Howard Straub, III[4], and Paul Bartlett[5]

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The goal of research and Extension around Bovine Leukemia Virus (BLV) is to use the knowledge gained to improve control and even achieve eradication of BLV from cattle herds. This project is about facilitating the end game: in herds and in the industry. We identified eleven Michigan dairy herds with a total of around 2500 cows from previous BLV projects as likely having low or no prevalence of the virus. Herd owners agreed to participate, and ELISA tests were conducted on all cows (milk or serum samples). Results of those were used to facilitate second stage testing using the CentralStar SS1 PCR test to determine the proviral load in the sample. All results were communicated to herd owners who were also provided with consultation on use of results in management decisions. These actions help herd owners with the endgame for BLV in their herds. One aspect of facilitating the endgame for the industry is the development of a low-cost monitoring system for herds identified as BLV-free. CentralStar has developed a protocol for bulk tank testing of dairy herds that is proving to be accurate in preliminary tests. Beyond that, conversations have been initiated about how BLV-free status can be recognized by the state and by dairy cooperatives without creating negative implications for the industry. This may be the greatest challenge.

# Practical BLV Eradication Measures for Commercial Dairy Farms

Donald Niles[*], DVM

*Co-owner and manager, Dairy Dreams LLC, Casco, WI*

The presentation is intended to provide the dairy veterinarian with a process by which Bovine Leukemia Virus can be eradicated from a dairy herd. The article will cover both biosecurity measures to limit horizontal transfer of BLV within a herd as well as testing procedures to identify BLV positive animals and sort by their likelihood of infecting others in the herd. There will also be a discussion as to why BLV-free status may become more desirable in our modern society, and how the large animal veterinarian is well positioned to lead eradication efforts.
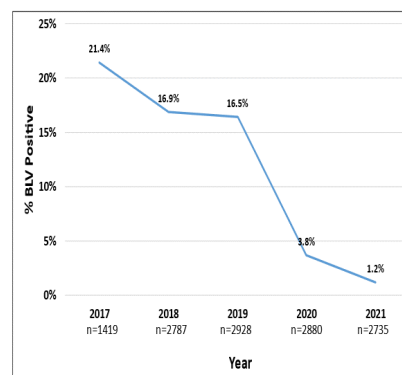
Dairy Dreams heard BLV prevalence as measured by number of lactating cows ELISA-positive divided by total cows tested has decreased with time. The total number of cows tested is indicated with each year.
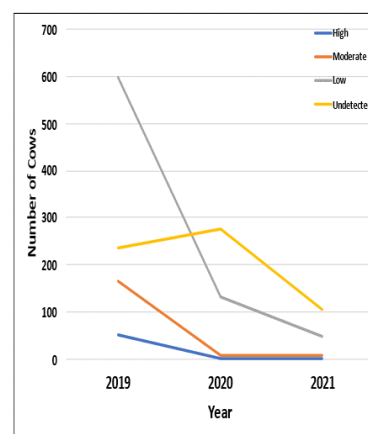
Dairy Dreams Proviral Load by Category with Time
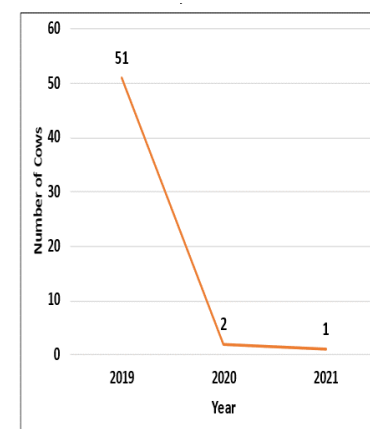
Dairy Dreams Number of High Shedders (PVL > 1000) Over Time

Team Updates

NEW MSU BLV Research Team Website

CANR.MSU.EDU/BLV

Conference Grant Proposal Committee

We want your help in drafting a conference grant proposal to host an International All Things BLV Conference!

If interested, please contact Tasia Kendrick (taxistas@msu.edu).

Thank You

Thank you for attending the 6th Annual All Things BLV Meeting. We look forward to seeing you next year!

BLV Research Team
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