Schedule & Abstract Booklet

October 24, 2019

162 Food Safety and Toxicology

Welcome to the 2019 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.

Any questions or meeting needs can be sent to:

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4:00 pm - on	Bartlett's Well Wishes into Emeritus Status Social (*Harrison Roadhouse, 720 Michigan Ave., East Lansing, MI*)

Impact of Bovine Leukemia Virus on Lymphocyte Counts and ELISA Status Across a Lactation Cycle in Dairy Cattle

C. Kellogg[*]*, B. Norby, P. Bartlett, P. Coussens, R. Erskine, D. Grooms, T. Byrem, and L. Sordillo*

Bovine leukemia virus (BLV) is a delta-retrovirus which primarily infects the B lymphocytes of cattle. An estimated 46% of all U.S dairy cattle are infected with BLV and ~30% of all BLV infected animals develop a persistent lymphocytosis. Our objective is to observe and document changes in BLV antibody levels, lymphocyte counts, and new infections over a lactation period in dairy cattle naturally infected with BLV to help determine critical time points for new infections within a herd. Two cohorts of 44 animals each were enrolled 150 days prior to calving. Enrollment consisted of animals scheduled to be dried-off within the same 7-day period and any heifers which would calve at the same time. 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. Complete blood counts were run every ~4 weeks from enrollment to ~60 days post parturition and then once after peak milk production. 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.

Evaluation of Two Blood Leukocyte Tests for Use on-Farm

H. Hutchinson[*]*, C.L. Swenson, T. Taxis, B. Norby, P.C. Bartlett*

Ongoing bovine leukemia virus (BLV) intervention field trials rely on blood cell differential (BLD) assays to assess animal health. Our research team currently utilizes two on-site analyzers: Advanced Animal Diagnostics QScout BLD system (QScout) and Oxford Science Inc. Genesis Hematology System (Genesis). The objective of this study was to compare the performance of both machines to the ADVIA 2120 hematology analyzer (ADVIA) at Michigan State University's veterinary diagnostic laboratory. Blood samples were collected from 157 dairy cattle on 3 commercial dairy farms. Samples were transported on ice to the diagnostic lab where they were analyzed on both QScout and ADVIA. Samples were then packaged for overnight shipment to Wisconsin to be analyzed using Genesis. Concordance of cell counts performed on QScout and Genesis were compared to ADVIA using Lin's concordance correlation. The presence of fixed and proportional biases was assessed using major axis regression and Bland-Altman plots. Results from QScout showed concordant total leukocyte (TLC) measurements; overall, constant or proportional biases were not identified for TLC. However, QScout overestimated lymphocyte (LC) by approximately 1,200 cells (95% CI: 1,100 to 1,400), resulting in discordant measurements with both proportional and constant biases. The TLC and LC counts measured from Genesis were discordant due to a strong proportional bias within the observed interval; Genesis increasingly underestimated TLC and LC as cell counts increased. However, within the normal reference interval (<11,800 TLC; <7,400 LC) proportional biases were not observed for either TLC or LC but a constant bias of approximately -1,200 TLC (95% CI: -2,100 to -240) was observed for TLC. The respective deviances from the ADVIA observed for both QScout and Genesis hinders the ability to justly compare the two machines. Insight to the discrepancies between the 2 on-site machines to ADVIA can inform ongoing field trials for necessary adjustments to on-farm blood cell differentials.

Longitudinal Changes in the Proviral Load and Lymphocyte Counts in Cows Naturally Infected with Bovine Leukemia Virus

H. Hutchinson, V.J. Ruggiero, B. Norby, K.R.B. Sporer, P.C. Bartlett*

Control programs aimed at reducing bovine leukemia virus prevalence (BLV) and incidence have shown success through removal of infected cattle with high proviral loads (hPVL) and high lymphocytes (hLC). Success of these programs can be enhanced by a greater understanding of BLV disease progression that may facilitate the early identification of infected cattle that which will progress to hPVL and hLC disease states. The objective of this study was to gain insight to disease progression by describing longitudinal changes in BLV diagnostic measurements. This analysis used a database from a two-year long intervention field trial conducted in three commercial dairy herds; results from whole-herd ELISA testing and the WBC differentials and PVL for BLV-ELISA positive cows were utilized. The range in LC per ul was 1,800 to 24,000. Median change in LC between two sample points was 0 LC per ul (IQR: -1,000 to 1,300, min: -8,800, max: 10,600). While an overall increase in lymphocyte counts was observed in 56% of cattle, results of a linear mixed model did not find a significant increase in lymphocyte counts overtime. The range in PVL was 0 to 180,000 copies per 100,000 cells. Median change in PVL between two samplings was 0 proviral copies (IQR: -2,200 to 7,200, min: -50,000, max:116,000). A linear mixed model found a significant increase of 3,000 proviral copies for each six-month sampling interval of positive cows. These results indicate that a majority of BLV-infected cattle experience relatively minor changes in LC and PVL overtime. Further analysis of cow-level factors associated with the major fluctuations may enrich ongoing control programs.

Example of On-Farm Complete Blood Count Test as a Management Tool for Assessing and Promoting Herd Health

A. Luttman, D. Wojciechowski*, T.M. Taxis, P.C. Bartlett*

Complete blood count (CBC) tests provide a summary of an animal's overall health. In particular, lymphocyte count (LC) can be indicative of infection, such as Bovine Leukemia Virus (BLV) amongst others. Companies such as Advanced Animal Diagnostics (AAD) have developed CBC machines that are convenient for on-farm testing. Here we will present on data from Pagel's Ponderosa, a Wisconsin dairy with a 6,000+ milking herd, who have been utilizing AAD's QScout ® BLD to assess herd health. By incorporating lymphocyte data from the CBC test into management decisions, Pagel's Ponderosa is able to actively segregate and remove high health-risk cows from the herd; thereby promoting overall herd health. An initial whole herd sampling was completed December 3-10, 2018, which involved collecting blood samples on all cows and analyzing the LC using AAD's QScout. Cows were then retested as they transitioned out of the fresh cow pens. Cows that tested with a LC between 10,000 and 14,999 were segregated and terminally bred to beef bulls. Cows tested with a LC over 15,000 were segregated and became a top priority to cull at dry-off. Data was analyzed quarterly by categorizing cows based on LC and reporting on whole herd distribution, lactation trends, and changes in LC since prior testing. By implementing management protocols at LC thresholds, Pagel's Ponderosa has decreased the percentage of the herd with high LC from 12.74% to 6.27% over a period of nine months. On-farm CBC tests can be an effective tool in herd health management; however, LC are not specific to BLV infection. An additional assay such as an ELISA is necessary to determine herd BLV prevalence. However, LC may be considered an indication of herd health. A decreased number of cows with high LC results in improved overall herd health.

Enhancing a sustainable dairy industry by controlling Bovine Leukemia Virus

T.N. DeJong[*], *C.L. Swenson, T.M. Taxis, B. Norby, C. Droscha, P.C. Bartlett*

Cattle infected with Bovine Leukemia Virus (BLV) have seriously altered immune systems, which contributes to their observed reduced milk production, shortened lifespan and predisposition to lymphoma. While over 21 countries have eradicated BLV by culling serologically positive cows, prevalence in the US has grown to approximately 45%, which costs producers $283 annually per milking cow. Therefore, we wanted to determine an integrated cost-effective method to eliminate BLV within a dairy herd. The study is being tested on a dairy farm that maintains approx. 3000 milking dairy cows. An initial whole herd sampling was completed in Nov. 2018, which included collecting blood samples on all cows and analyzing them using the Genesis hematology analyzer on the farm. Any sample that tested with a lymphocyte (LY) count above the high cutoff line (10.0 K/μL) was shipped to CentralStar in Lansing, MI to have serum ELISA and qPCR-(SS1) tests ran. Each sample was then reported with either a negative or positive value for serum ELISA and a proviral load value based on SS1 test (copies of BLV/μL of blood). Following the whole herd scan, cows were then sampled as they came fresh, all tested on the Genesis analyzer to determine LY count, and sent for ELISA testing. All ELISA positive samples were then SS1 tested. The farm is currently using this protocol to prioritize segregation and culling of all BLV positive cows and will continue to do so until all samples test negative for ELISA. Using this protocol, the farm has been able to reduce BLV prevalence in the herd over the first 6 months of the study by reducing the percentage of high LY cows from 4.22% to 1.42%. By the one-year mark, the whole herd will have an ELISA (and if applicable an SS1) test on file, which will continue to help the farm remove BLV positive cows. Current herd BLV measures will be discussed at the presentation of this study. To reduce BLV transmission, an integrated testing system is being used to identify the most infectious cattle for segregation and culling.

An Attenuated Vaccine That Efficiently and Persistently Protects Against Bovine Leukemia Virus in Herds

L. Willems[*]

There is currently no efficient vaccine that protects against bovine leukemia virus (BLV) infection. We now propose a novel approach based on the use of a recombinant live-attenuated BLV provirus. The rationale behind this strategy relies on the deletion of genes required to induce pathogenesis maintaining the integrity of those involved in infectivity. We have identified a BLV attenuated provirus that is infectious but replicates at reduced levels in cattle. Vaccinated animals resist challenge by a wild-type BLV virus in experimental conditions. The vaccine can be delivered by conventional DNA injection or a BHK21 cell line carrying a stably integrated vaccine. Large-scale vaccination trials are currently ongoing in Argentina. The attenuated strain elicits a strong anti-BLV immune response and does not spread to uninfected sentinels maintained during 10 years in the same herd supporting biosafety of the vaccine. Passive antibodies are transmitted to newborn calves via maternal colostrum and persist during several months. Nevertheless, the BLV attenuated provirus does not transmit from cows to calves. The vaccine provides sterilizing immunity in settings of commercial dairy herds.

In summary, we have identified a safe BLV attenuated provirus with impaired transmissibility that efficiently protects against infection in herd conditions.

Constructing a Synthetic Peptide Vaccine Against Bovine Leukemia Virus

S. Chugh and X.Huang*

Bovine Leukemia Virus (BLV) is a C-type retrovirus of cattle causing enzootic bovine leukosis. It is a major animal health problem spread around the world causing huge economic losses. The National Animal Health Monitoring System (NAHMS) determined that BLV is present in 89% of US dairy operations.[1] The overall result of the infection causes reduced milk production, and reduced life-expectancy and lymphoma. The estimated loss to the dairy industry is more than $42 million annually.[1]

The goal of our project is to develop a synthetic peptide vaccine against BLV, which is able to overcome the current bottlenecks of low immunogenicity and rapid clearance from the host's immune system. The peptide antigen used is derived from the envelope protein gp51 of BLV **(Fig.)**. The membrane glycoprotein gp51 and gp30 are directly involved in virus infectivity and gp51 is the first viral antigen to elicit immunological response in the infected hosts. The most accessible and potent peptide antigenic sequence derived from env. gp51 was chosen for this study, i.e. residues 177-192 (PDCAICWEPSPPWAPE oxidized to form a disulfide bond between two cysteine residues).

The peptide antigen was conjugated on an icosahedral virus like particle, Qβ in order to be displayed in a highly organised manner. Two approaches for chemical conjugation were explored, one using a homobifunctional nitrophenyl ester linker and another one by exploiting the isothicyanate chemistry. This vaccine construct, when administered in a mouse model, elicited high anti-peptide IgG titers. The polyclonal antibodies were also able to recognise and bind to the gp51, the whole BLV particle through ELISA test, and immunogold labelling respectively.

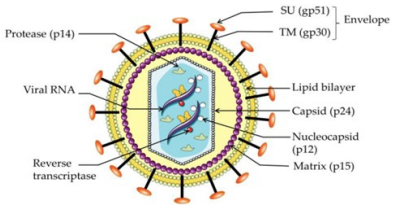

Fig. Schematic representation of the viral particle of Bovine leukemia virus (BLV)[2]

[1]USDA-APHIS, VS, NAHMS 1996 Dairy Info Sheet
[2]Da, Y.; Shanks, R. D.; Stewart, J. A.; Lewin, H. A. Milk and Fat Yields Decline in Bovine Leukemia Virus-Infected Holstein Cattle with Persistent Lymphocytosis. *Proc. Natl. Acad. Sci.USA.* **1993,** *90* (14), 6538-6541.

Sequence-based Typing of MHC BoLA Alleles to Determine BLV Host Resistance

C. Lohr, K. Sporer, V. Ruggiero, C. Droscha, P. Bartlett*

Bovine leukemia virus (BLV) is highly prevalent in U.S. dairy cattle and is an economic burden where about 30% of BLV-infected cattle develop lymphocytosis due high proviral load (PVL), leading to immune dysfunction, reduced milk production, and premature culling. Bovine leukocyte antigen (BoLA), the major histocompatibility complex II (MHC), is vital for infectious disease resistance in cattle. Exon two of the II BoLA-DRB3 and DQA1 alleles' encodes the extracellular portion of the molecule and is polymorphic in nature to provide antigen-presenting cells with variability in immune response to particular pathogens for individuals. Our objective is to investigate exon two of the BoLA-DRB3 and DQA1 alleles in the context with BLV PVL in U.S Holstein cattle as well as determining BLV viral type for each animal. Cows with persistently high PVL and LC phenotypes and those with disease that have not advanced were identified in our ongoing, seven herd, 3000 cow BLV field trial. Exon 2 of the BoLA-DRB3 and DQA1 alleles were analyzed via end-to-end amplicon sequencing in a 2x250bp paired end format using a MiSeq v2 500 cycle flow cell. Trimmed reads were aligned to the reference haplotype sequences for BoLA, DRB3 and DQA1, obtained from EMBL-EBI, and genotypes for each allele were determined by counting the frequency of reads mapping to each reference haplotype sequence. The BLV-envelope gene is also being sequenced via Sanger sequencing within selected animals to determine viral type and pathogenicity. Candidate alleles were identified from two sets of cattle, an initial set of 91 (57 high, 34 low) animals, and a secondary set of 95 (64 high, 31 low) animals. The two sets of sequencing suggest that BLV resistance is associated with DRB3 alleles *0601, *0902 and *1701 and DQA1 alleles *0301 and *0204, and that BLV susceptibility is related to DRB3 alleles *0101, *1101, *1501 and DQA1 alleles *0101 and *1401. Our data agrees with previously stated associations between BLV resistance and DRB3*0902, *1701 and DQA1*0204, as well as BLV susceptibility and DRB3*0101,*1101 and *1501.

MiRNA Extraction from Milk as a Diagnostic Tool for Bovine Leukemia Virus Identification and Evaluation

*J. Zenchak, K. Kesler, K. Sporer, T. Byrem, C. Droscha**

The prevalence of bovine leukemia virus (BLV) has now surpassed 40% in US dairy cattle, with over 80% of US dairy herds infected. Although BLV is often asymptomatic, the virus is known to cause immunosuppression, reproductive inefficiency, and decreased longevity. Current diagnostics for BLV include ELISA, to determine presence of anti-BLV antibodies, and qPCR, to determine the relative number of copies of BLV provirus to host DNA (proviral load, PVL), enabling stratification of infectiousness. Both tests require a blood sample, of which collection is invasive and labor-intensive, prohibiting scalability to national levels. Milk samples offer a more convenient alternative to blood, however, BLV does not migrate to the mammary gland so PVL cannot be determined using milk. We sought to identify a novel target in milk indicative of BLV status and PVL. MicroRNAs (miRNAs) are emerging biomarkers, and BLV is known to produce several miRNAs that are critical for effective viral function. BLV miRNAs have been reliably detected in blood and correlate with PVL. The purpose of this study was to determine whether BLV miRNAs can be reliably detected in milk and correlate with PVL. Blood and milk samples were obtained from a local cooperative herd. Herd BLV status was analyzed using ELISA and qPCR assays; samples were stratified and identified based on PVL. MiRNA extractions were performed using our optimized protocol for 96-well plate automated extractions. PCR analyses were performed using Reverse Transcriptase and TaqMan assays. MiRNA extraction methods were optimized for milk, and an endogenous miRNA control was identified (148a). BLV miRNAs (B5-5p, B3-3p, and B1-3p) were found to significantly correlate with PVL in whole blood samples. Milk-derived BLV miRNAs are currently being assessed in a range of PVL-verified cows, from negative to asymptomatic to advanced stage BLV. The purpose of this study was to determine whether BLV miRNAs can be detected in milk due to ease of sample access, decrease invasiveness, and decreased cost for sample collection/testing. Increased testing and knowledge of BLV status within herds will enable producers to make more informed decisions about animals in their herd, and could lead to decreased rates of BLV nationwide.

Identification of Epigenetic Markers Predictive of Late Embryonic Mortality in Bovine Milk

K. Kesler, J. Zenchak, K. Sporer, T. Byrem, C. Droscha*

Reproductive efficiency is the most important factor in determining producer profitability. Yet, embryonic mortality contributes 56% of reproductive failure. Industry standards of pregnancy diagnostics include transrectal palpation, ultrasound and measurement of pregnancy-associated glycoprotein (PAG) levels via ELISA; however, no diagnostic can predict late embryonic mortality (LEM), one major contributor to reproductive failure. Developmental processes and epigenetic factors tightly control the timing and magnitude of gene expression. Circulating epigenetic factors such as microRNAs (miRNAs) to determine embryogenic fidelity is at the forefront of modern molecular diagnostics as in non-invasive prenatal testing. Circulating miRNAs in serum and milk are reliable non-invasive biomarkers of animal physiology due to their stable, sensitive, and specific nature. We hypothesized that LEM-specific miRNAs in milk are present, predictive and robust biomarkers of embryonic mortality. Milk represents a non-invasive and economical diagnostic medium for producers. This work will aid in the discovery of milk-based biomarkers predictive of LEM equipping producers with a novel diagnostic that delivers enhanced knowledge about pregnancy status empowering them to make more profitable breeding decisions. MicroRNAs are extracted using an optimized semi-automated protocol and miRNAs are verified using RT-qPCR. Candidate miRNAs are being profiled via RNA -seq and validated using RT-qPCR. MicroRNA-148a and miR-26a were characterized as potential endogenous controls due to their uniform presence during the lactation cycle. MicroRNA-222 and miR-25 were screened as LEM candidates as proof of principle of dynamic physiologic targets. Physiologic miRNAs are present and informative in milk. Discovery of milk based epigenetic biomarkers will create a platform for diagnostics that can deliver improved knowledge about cow physiology. This work is contributing to the development of a new paradigm in dairy diagnostics; reforming the value added to a milk sample for dairy producers and practitioners.

BLV Super-Shedders: Insights Into a Longitudinal Field Trial

K.R.B. Sporer, C.J. Droscha, T.M. Byrem, P.C. Bartlett*

It is now well established that bovine leukosis, caused by infection with bovine leukemia virus (BLV), causes far-reaching effects on cow overall health and performance. Management interventions to reduce transmission have not proven effective at decreasing prevalence. Recent improvements in BLV diagnostics have aided in the identification of "super-shedders," cows with a high proviral load (PVL) that are responsible for the majority of transmission in the herd. These highly infectious cows are a critical control point for reducing within-herd prevalence. The objectives of the current study are to employ a longitudinal field trial to gain insight into the dynamics of BLV prevalence and incidence in various herds and to then develop strategies to support dairy producers in reducing the effects of BLV infection in their herds.

Our field trial, employing our qPCR "SS1" assay, is currently in its second year with over 3,000 cows enrolled in 7 herds with varied size, production, and management practices. Twice yearly, whole-herd ELISA tests are performed using Dairy Herd Information (DHI) test milk samples to establish current BLV prevalence. Whole blood is then collected from ELISA-positive cows within four weeks, genomic DNA extracted, and SS1 qPCR assays performed. Cows from each herd are ranked by descending PVL, and producers and veterinarians are consulted on results and management changes that may reduce the impact of the highest shedding cows.

BLV ELISA and SS1 results, as well as custom reports with each herd's goals in mind, have been presented to producers for three time points to date; testing and sample collections are still in progress. An ongoing repository of genomic DNA from total leukocytes from all enrolled cows continues to accumulate and can be queried for future studies.

We have acquired new insights into the productive life of "super-shedders," BLV-positive cows that are non-shedders, and the incidence in each enrolled herd as well as challenges in managing this disease in the current dairy economy.