Bovine Leukemia Virus in the U.S.: Impact and Options for Control

#1 What is it?
Why does it matter?
What Options for control?
Where to start?
What can USAHA do?

Visit our website at WWW.BLVUSA.com

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This material is based on work supported by the United States Department of Agriculture and the National Institute of Food and Agriculture award numbers 2014-67015-21632, 2014-68004-21881 and 2015-67028-23652
BLV Group at Mich State U. - 2017

• Faculty
  – Paul Bartlett - Todd Byrem
  – Paul Coussens - Casey Droscha
  – Ron Erskine - Lorraine Sordillo
  – Phil Durst - Dan Grooms

• Graduate Students
  – Oscar Benitez - Meredith Frie
  – Vickie Ruggiero - Chris Kellogg
  – Rebecca LaDronka
  – Holden Hutchinson

• Industry
  – Todd Byrem (NorthStar Coop)
  – Casey Droscha (NorthStar Coop)
Why talk about BLV?

- I was taught in school that BLV is not a problem.
- We’ll see that our survey of 103 producers indicated that only 10% view BLV as a “significant problem”.
- Why did USAHA give me 45 minutes? And why are you here?
- Because some things have changed and some things have just been discovered.

- I’ll post slides on [WWW.BLLVUSA.com](http://WWW.BLLVUSA.com)
## NorthStar Cooperative BLV ELISA Testing

<table>
<thead>
<tr>
<th>Fiscal Yr</th>
<th>Blood ELISA</th>
<th>Milk ELISA</th>
<th>Reagents sold</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>1,294</td>
<td>8,520</td>
<td>12,960</td>
</tr>
<tr>
<td>2016</td>
<td>8,987</td>
<td>18,037</td>
<td>16,848</td>
</tr>
<tr>
<td>2017</td>
<td>5,404</td>
<td>24,987</td>
<td>25,488</td>
</tr>
</tbody>
</table>
What is it?

Clinical Presentation
Prevalence
Immune suppression
Bovine Leukosis:

• Bovine Leukosis or Enzootic bovine leukemia (The disease)

• Bovine Leukemia Virus (BLV) (The agent)
  – Retrovirus like HIV/AIDS or HTLV in people
    • Similar transmission and immune disruption.
  – RNA virus invades blood lymphocytes and integrates into the DNA as a provirus

Current Understanding

Green cells: non-infected B-cells;

Red cells: infected B-cells harbouring BLV provirus;

Black cells: transformed tumour cells harbouring BLV provirus.

Adapted from: Luc Williams. Scientific Opinion on enzootic bovine leukosis, EFSA Panel on Animal Health and Welfare (AHAW) European Food Safety Authority (EFSA), Parma, Italy 2014
Transmission

- Transmission primarily through transfer of provirus-infected B lymphocytes
- Mammary epithelial cells, T-cells lymphocytes and maybe other types of cells can also be infected.
  - Their importance in transmission is unknown.
- Free RNA virus is fragile and doesn’t last long in the environment
  - Their importance in transmission is unknown, but it is important for many other retroviral diseases.
BLV Prevalence in the U.S.

Cow Prevalence in U.S. herds:

- 1975  < 10% of dairy cows infected
- 1988  ~ 28%
- 1996  ~ 41%
- 2010  ~ 33% (Michigan)
- 2017  ~ 43%

Source: USDA (NAHMS), USDA-APHIS, 1999; USDA-APHIS, 2008
BLV Prevalence in the U.S.

• In the 1960s and 1970s, the U.S. and Canada decided that control of BLV was not cost-effective.
  – Lymphoma/lymphosarcoma was the only concern at that time.

• Bovine leukemia virus has been eradicated from all cattle in at least 21 nations.
  – Achieved by testing and culling the antibody positive animals.
  – But they were starting with very low prevalence usually < 5%.
Don’t feel locked in.

Feel free to roam around to get coffee or whatever.
BLV-infected cattle showed a reduced response to the J5 vaccine

Erskine et al., Vet Med Intl, 2011
Methods: 2010 BLV Study (Erskine)

Stratified (herd size) random sample of Michigan DHI herds
Milk collected by DHI was “tagged” to also be tested for BLV by the milk ELISA test.

A Herd BLV Profile tested the 10 most recently fresh cows from each of four lactation groups

10 cows from lactation 1
10 cows from lactation 2
10 cows from lactation 3
10 cows from lactation ≥ 4

The mean prevalence for all herds was 32.8%, with means of 18.5, 28.8, 39.2, and 44.8% of 1st, 2nd, 3rd, and ≥4th lactation animals infected, respectively. Ave. herd size: 408 cows on test.

Distribution of within-herd BLV prevalence
113 MI dairy herds: 2010

15 herds were totally negative

Why are the 15 negative herds significant?

• It means that negative herds can be maintained even if most neighboring farms are infected.
  – And note that the NAHMS study found that 17% of dairy herds were BLV-negative.
• This indicates that once a closed herd eradicates BLV, it should be possible to keep BLV out.
2015-2016 National study: Enrollment

- 40 cows tested in each herd
- 103 herds
- 10 most recently fresh (>10 DIM) in each of the 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}, & 4\textsuperscript{th} and greater lactation groups
- 4,120 cows total

Prevalence:
- Herd Profile: 46.5%
- Weighted avg: 42.4%
Immune disruption (Sordillo, Coussens, Frie in 2016 and 2017)

**BLV+ B cell Immunity**
- Antigen-specific IgM
- Antigen-specific IgG
- CD5^{dim+} circulating cells
- CD45R0^{+} circulating cells
- Antigen-specific activation

**BLV+ T cell Immunity**
- αβ CD45R0^{+} circulating cells
- γδ CD45R0^{+} circulating cells
- αβ IFNγ production
- γδ IFNγ production
- αβ IL4 production
- γδ IL4 production

• Other Retroviruses, like HIV/AIDS, are known to cause immune disruption.
Bovine Leukemia Virus in Dairy Cattle:

#1 What is it?

#2 Why does it matter?

What Options for control?

Where to start?

What can USAHA do?

Dr. Paul Bartlett, MPH, DVM, PhD, ACVPM-Epidemiology, Professor. College of Veterinary Medicine, Michigan State University, E. Lansing, MI USA

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Why Does it Matter?

• Economic Impact
  – Tumors
  – Lost milk production
  – Shortened cow longevity

• Animal Welfare

• Public health?

• Regulatory burden
  – Embryo transfer
  – Breeding Bulls
  – Exports

See our website at WWW.BLVUSA.com
Tumors occur in about 1-5% of infected animals.

Lymphoma costs the U.S. dairy industry more than $16 million annually.
Impact of BLV on milk production

- Three large databases of > 100 herds each have shown a decrease in milk production associated with BLV infection.
  - A USDA NAHMS study in 1996 showed that BLV was associated with reduced milk production.
  - Michigan herd-level analysis in 2010 provided further evidence of decreased milk production associated with BLV.
  - 2015-2016 national study of 103 herds in 11 states (Mich State U.)

Figure 2. Association between herd prevalence of bovine leukemia virus and rolling herd average milk production (NAHMS USDA, 1997; Ott, 2003; Erskine, 2012; LaDronka, 2017)
Table 3- Final model for associations between herd bovine leukemia virus index and herd level variables on rolling 12 month herd average milk yield in 104 Michigan Holstein dairies. 
\[ R^2 = 0.375; \text{Residuals normally distributed (Shapiro-Wilk } W = 0.99); n=104 \text{ herds} \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>13,648</td>
<td>299</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BLV Profile</td>
<td>-1,397</td>
<td>402</td>
<td>0.0008</td>
</tr>
<tr>
<td>Milking twice per day</td>
<td>-1,049</td>
<td>186</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SCC</td>
<td>-5.38</td>
<td>1.36</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
2010 cow-level analysis also showed BLV infection was associated with lost milk production

- BLV ELISA Antibody Test
  - MEAN BLV (neg) 11,497kg
  - MEAN BLV (pos) 11,378kg

  1% difference in 305 ME milk production

- BLV ELISA Antibody Test
  - Negative ELISA 11,502kg
  - Low positive 11,459kg
  - Medium positive 11,453kg
  - High positive 11,148kg

  Difference: neg. vs. low: 0.4%
  Difference: neg. vs. high: 3.1%

Impact of BLV on **Cow Longevity**, i.e. how long the cows stay in the milking herd.

Sources:


In 2010 we noticed an association between herd BLV prevalence and the proportion of the herd in the > 3rd lactation.

- Herds with higher BLV prevalence tended to have younger cows
- Suggesting that cows were not lasting as long.

![BLV Profile (Prevalence)](image)

\[
r = -0.286; P = 0.0021
\]
Follow-up study

• We followed the records of the cows in the 2010 study to see if (and when) they died or were culled.
• This was 3,849 Holsteins in 112 Michigan dairy herds followed for an average of 597 days (1.6 yrs) following testing for BLV antibodies with an ELISA milk test.
• We compared BLV-positive cattle to their BLV-negative herd mates.
  – Cows sold for dairy purposes were excluded.
Survival Analysis

• The hazard ratio of 1.23 (p < .0001) indicated that BLV positive cows were 23% more likely than their BLV-negative herd mates to die or be culled during the 597 day (1.63 yrs) monitoring period.
Survival Analysis of BLV-positive versus BLV-negative Dairy Cattle

Day of BLV test

Survival in days post testing

100% at start

About 57% left

About 43% left

BLV Neg

BLV Pos
Survival in days post BLV ELISA testing

Survival in days post BLV testing
## BLV ELISA Milk Test category and cow longevity

<table>
<thead>
<tr>
<th>OD category</th>
<th>% of cows</th>
<th>Likelihood of leaving their herd</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1 (neg)</td>
<td>66.2%</td>
<td>-</td>
</tr>
<tr>
<td>0.1-0.24 (low pos)</td>
<td>10.6%</td>
<td>8%</td>
</tr>
<tr>
<td>0.25-0.5 (med pos)</td>
<td>13.6%</td>
<td>25%</td>
</tr>
<tr>
<td>≥0.5 (high pos)</td>
<td>9.6%</td>
<td>40%</td>
</tr>
</tbody>
</table>
Looking only at animals which started their monitoring period in their first lactation

Both the milk production analysis and the cow longevity analysis suggest that first lactation animals show little ill effect of BLV infection.

(Remember that progeny testing for AI sires is done almost entirely on first lactation performance.)

No difference between BLV Pos and BLV Neg
Mastitis and BLV status
Bo Norby, Lorraine Sordillo

• Studied cows in the transition period
• Cows that were ELISA+ and had LC ≥ 10,000 at dry off were 5 times more likely to develop mastitis as compared to all other cows
  – RR=5.0 (1.6-15.9), p=0.006, n=271
• Lameness also at a higher rate in ELISA+ (but not significant (yet).
BLV and higher SCC
Dr. Rebecca LaDronka

• 9 of 103 herds evaluated so far
  – ELISA-positive cows were 1.64 (1.016-2.661) times more likely than their negative herdmates to have at least one new case of mammary inflammation.

• With 16? of 103 herds so far
  – Odds ratio .94

• So – We’ll wait until all 103 herds are done.
“49.1% of dairy cattle (964/1,963) from 6 provinces of China and 1.6% of beef cattle (22/1,390) from 15 provinces were BLV positive”

“The BLV-positive cows had significantly lower milk production in the early (26.8 vs. 30.9 kg) and middle stages of lactation (22.2 vs. 26.1 kg) in animals with ≥4 parities than the BLV-negative cows; they also had significantly higher SCS in early and middle lactation stages (early = 5.2 vs. 4.3; middle = 4.9 vs. 3.9) in animals with ≥4 parities.”

“Vaginal secretions and feces may be involved in BLV transmission” (PCR evidence)
Canadian study

- Cows with BLV were more likely to be culled.
- BLV-positive cows had lower life-time milk production
- Recommend a national control program.

Economic Impact

2015-6 Nat’l Survey: When asked “How much of a problem do you believe BLV is for your herd?”, 50% of dairy producers from 112 herds thought that BLV was not a problem at all.

An additional almost 40% thought that BLV was a small problem, but not significant,

Only ~ 10% who thought that BLV was a significant problem.

Lack of precise information regarding the costs has discouraged researchers from making rough estimate of the total cost of BLV infection.

Evidently many producers were assuming that lack of an estimate meant that the estimate is $0.

However, a rough estimate may be better than no estimate at all.
The W.K. Kellogg Biologic Station Pasture Dairy Center [http://pasturedairy.kbs.msu.edu/](http://pasturedairy.kbs.msu.edu/) started on one of our field trials.

At the start of the program in 2015, we estimated how much BLV was costing this farm.

- Milk loss and poor survival were based on our 2010 study results.
Inputs: Enter your herd's data in the blue highlighted boxes below

Number of animals in your milking herd: 140

Percentage of your milking animals that are ELISA positive for BLV on milk or serum test: 62.00%

Average slaughter value of a cow from your milking herd: $1,260

Value of an average animal in your milking herd. Think of the lowest price you would accept to sell an average cow from your milking herd: $2,250

In the last 12 months, how many of your cows were condemned at slaughter for lymphoma so that you received nothing for the sale? 2

Rolling herd average milk production - pounds per cow per year: 21,000

Price of milk - per cwt: $17.10

Any additional cost to control BLV: supplies, testing, etc. $1,000.00
### Outputs: In the yellow highlighted boxes

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of decreased longevity per year for your entire herd</td>
<td>$12,083.74</td>
</tr>
<tr>
<td>Condemnation loss per year for your herd</td>
<td>$2,520.00</td>
</tr>
<tr>
<td>Milk production loss</td>
<td>$37,584.94</td>
</tr>
<tr>
<td>Any additional cost to control BLV</td>
<td>$1,000.00</td>
</tr>
</tbody>
</table>

**Total: Estimated yearly herd BLV impact (your herd)** $53,188.68

**Total: Estimated yearly herd BLV impact per 100 cows** $37,991.91

That’s $380, per milking cow each year.

See our website: [www.BLVUSA.com](http://www.BLVUSA.com)
Impact of BLV on International trade, ET and raising bulls for AI

• Export of US dairy cattle and cattle products may become increasingly difficult as other countries attempt to:
  – maintain their BLV-free status or
  – implement BLV eradication programs.
Evidence of Human Health Effects of BLV?

- No epidemiologic evidence of adverse human health effects of BLV.
- Antibodies to BLV proteins are relatively common in people.
- BLV can be grown in human tissue culture cells

These last two observations were made after the 1960’s when the U.S. decided that BLV was not worth controlling.
Two research teams have found genes of BLV origin in human mammary cells.

These two research teams have found conflicting evidence regarding whether genes of BLV origin are more often found in cancerous human mammary cells or in non-cancerous human mammary cells. (Buehring, 1997, 2001 & 2003; Giovanna, 2013)

Australian study: “BLV DNA found in breast tissue of 40/50 (80%) of women with breast cancer versus 19/46 (41%) of women with no history of breast cancer” (Buehring, 2017)

We do know that BLV infects the mammary cells of cows.

Clearly - further work is needed.
Is BLV an Animal Welfare Issue?

• HIV/AIDS in people is probably a good model of the clinical syndrome of BLV in cattle

• Longevity is a widely accepted measure of human welfare.
  – Years of Potential Life Lost (YPLL) and average Life expectancy are well-established measures of human wellbeing.
Should cow longevity (life expectancy) be recognized as measure of animal welfare?
Judging from human HIV/AIDS, these BLV animals probably suffer toward the end of their lives.

The ideal life for a food animal is one that is comfortable, healthy and free of pain until the “lights are instantly turned off” when they are humanely stunned and slaughtered so that their bodies can become our food.

This graph is consistent with immune suppression, chronic debilitation and animal suffering.
Consumer Perception of BLV Infection

Public Health and Animal Welfare

• Perception is everything.

• It could be difficult for dairy spokespersons to convince skeptics that:
  – U.S. cows are not slowly dying of cancer
  – that cow milk is not full of cancer cells
  – and that it is acceptable to have bovine leukemia virus inside our human mammary cells.

• Consumer reaction to BLV could seriously damage the sustainability of the U.S. dairy industry in a global market where many other nations have made BLV control a priority.

• Would consumers be interested in milk from BLV-negative cows as a “premium“ specialty product?
Bovine Leukemia Virus in Dairy Cattle:

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What other countries did about BLV

**Twenty-one countries where BLV is Currently Absent (Year Eradicated)**


**Countries Where BLV is Present, but does not Exhibit Clinical Signs or is Restricted to a Certain Area**

- Australia, France, Greece, Hungary, Italy, Latvia, Lithuania, Mexico, Moldova, Portugal, Germany*  
  *Germany is officially labeled as BLV free, but has had multiple recent cases.

Source: (OIE, 2013)

Accomplished by testing for BLV antibodies and culling the positives.
Possibilities for control of BLV in the U.S.

• Slaughtering all ELISA-positive animals is prohibitively expensive when starting with a high prevalence.

• So – what to do?
  
  Phase 1: Management changes and other actions to reduce BLV prevalence.
  
  Phase 2: Eventually cull remaining ELISA-positive animals to eradicate BLV from the herd.
  
  Phase 3: Once eradicated, maintain a closed herd and monitor to assure the herd remains free of BLV.
How to control BLV

• See our website at WWW.BLLVUSA.com

or

Options for the control of bovine leukemia virus in dairy cattle

Paul C. Bartlett, MPH, DVM, PhD; Lorraine M. Sordillo, PhD; Todd M. Byrem, PhD; Bo Norby, DVM, PhD; Daniel L. Grooms, DVM, PhD; Cheryl L. Swenson, DVM, PhD; Jessica Zalucha, BS; Ronald J. Erskine, DVM, PhD

JAVMA, Vol 244, No. 8, April 15, 2014
Why isn’t there a vaccine?

• It is difficult to develop vaccines for retroviral diseases
  – e.g. HIV/AIDS

• There have been many unsuccessful attempts to develop a vaccine for BLV.

• At least two groups are working on vaccines now.
  – One was a genetically engineered vaccine strain (gene deletant) which has decreased replication.
  – Bioengineered vaccines are generally “slow” to be approved in the U.S.
Selection of cattle for resistance? 

- Apparently all exposed cattle become ELISA-positive.
- Genetic factors may be important in determining if an infected animal progresses to:
  - persistent lymphocytosis
  - high proviral load
  - high infectivity to her herdmates.
- The genetic associations are not 100%
- Work has been done in Japan and Argentina to identify some alleles associated with resistance and susceptibility.
  -- Our Japanese collaborators found that about 1/3 of cows with “resistant” genes still get high PVL and high lymphocytosis
- Already being done by some AI firms
Genetic Sequencing

- Cows with at least 2 consecutive semiannual tests:
  - 57 high proviral load, high lymphocytosis ("super-shedder")
  - 34 "resistant"
- Next Generation Sequencing
  - Sequence DQA1.2 exon and DRB3.2 exon
  - Identify DQA1-DRB3 haplotypes and associations with PVL phenotypes
  - Look for single nucleotide polymorphisms (SNPs)
  - Sequence BLV provirus to identify BLV strain(s)
- Unfortunately - The major histocompatibility complex class I allele (BoLA-A) appears to be somewhat associated with both susceptibility to persistent lymphocytosis and with high milk production potential (Da et al. 1993).
Medical hygiene to control BLV

• **Field trial:** The role of needles & OB sleeves in transmission (in preparation)
  
  • Pre-trial anecdotal evidence was not encouraging.
  
  • Randomize BLV-neg cows into control group & intervention group
  
  • Tagged cows – got a new needle & new sleeve every time
  
  • Control cows – shared needles and sleeves with rest of herd
  
  • Compared the rate of new BLV infections 2x/year
Rate of new BLV infections among tagged cows (single use needles and sleeves) or controls (shared needles and sleeves)

<table>
<thead>
<tr>
<th>Sum Totals Fall 2014 to Fall 2015</th>
<th>Control</th>
<th>Tagged</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>244</td>
<td>262</td>
<td>506</td>
</tr>
<tr>
<td>pos/susp</td>
<td>40</td>
<td>53</td>
<td>93</td>
</tr>
<tr>
<td>neg</td>
<td>205</td>
<td>209</td>
<td>414</td>
</tr>
<tr>
<td>Rate of New Infections</td>
<td>16.4%</td>
<td>20.2%</td>
<td>18.4%</td>
</tr>
</tbody>
</table>

<p>| | | | |</p>
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<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Relative Risk</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% LCI</td>
<td>0.90</td>
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<td></td>
</tr>
<tr>
<td>95% UCI</td>
<td>1.90</td>
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</tr>
</tbody>
</table>
Medical Hygiene...?

Takeaway:

• In these herds, re-use of hypodermic needles and reproductive exam sleeves was not a major route of BLV transmission within the milking herd.

• But medical hygiene is still important as veterinarians need to be “above reproach”.
ELISA Test & Cull the Positives

The approach used by other nations to eradicate BLV

Criteria:

- Starting prevalence < 5%
- Closed herd
- Cull or segregate ELISA positive animals

3 enrolled herds:

- Michigan (2)
- Wisconsin (1)
### Preliminary Results - Test & Cull Field Trial (Vickie Ruggiero)

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<td></td>
<td>1</td>
<td>8</td>
<td>2.33</td>
<td>3</td>
<td>2.2</td>
<td>27</td>
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<tr>
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<td>5</td>
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<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
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<td></td>
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</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
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</table>

**Field trial:** Cull all ELISA-positives on milk test 1 or 2 times per year.

**Lesson learned:** Infected young stock will constantly re-infect the milking herd.
Selective Culling or Segregation of “Super-shedders”

• Proviral load (PVL) is a quantitative PCR measure of the number of copies of the DNA provirus per unit of blood.

• Two years ago Drs. Aida and Takeshima brought us their qPCR test to measure PVL. Takeshima returned in the summer of 2016.

• Cows with high PVL and lymphocytosis are called “Super-Shedders”.

• Maybe only the ~1/3 of ELISA-positives that are BLV super-shedders should be prioritized for culled or segregation (Alvarez, 2013; Gutiérrez et al., 2012)
This data is from a small Michigan herd in 2015 that had 12 BLV-positive cattle. All positive cattle were tested for proviral load (PVL).

We think that PVL may indicate how infectious the blood is, like viral load and/or proviral load does for other retrovirus.

<table>
<thead>
<tr>
<th>Ear tag</th>
<th>PVL</th>
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<tbody>
<tr>
<td>1</td>
<td>29.63</td>
</tr>
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<td>46,632.61</td>
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<td>12</td>
<td>48,826.43</td>
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</table>

14,000 to 50,000 is considered “moderate”
Evidence that PVL and lymphocyte count are measures of infectiousness to herdmates

- 20 low PVL cows introduced into a herd of 105 BLV ELISA-negative cattle.
- No transmission in the subsequent 20 months.
- He also notes in the discussion that it takes about 926 infected lymphocytes to infect sheep (Burny et al 1987) “Hence, the minimum BLV infective dose in [low PVL] cattle is contained in a volume of blood so great that it is unlikely to be transmitted between animals …”
- Also – viral load or proviral load is recognized as a measure of infectivity for other retroviruses.

PVL and lymphocyte count seen as measures of infectivity

- Identified BLV from different cows (finger prints).
- Cattle infected with less than 3 copies /100 cells (i.e. low PVL) did not transmit BLV to other cattle for more than 30 months.

Selective Culling of “Super-shedders”

• Maybe herd with high prevalence should prioritize BLV super-shedders for culling and segregation?
• PVL and lymphocyte count are correlated
• But PVL as measured by CoCoMo is:
  – Not licensed in the U.S.
  – Is laborious (1 grad student for 1 wk for ~100 cows).
• Based on our immunology studies, lymphocyte count is may be our best measure of the cow’s immune dysfunction and likely prognosis for staying in the herd.
• Based on the latest research, PVL may be our best measure of the cow’s infectivity to herdmates.

QScout® Advanced Animal Diagnostics

- Complete blood count with wbc differential.
- $18K machine
- $5/sample
- in 2 minutes

Lymphocyte count
correlation between proviral load and lymphocyte count

correlation 0.771

Dairy Cows
correlation between proviral load and lymphocyte count

correlation 0.771

Dairy Cows

![Graph showing the correlation between proviral load and lymphocyte count in dairy cows. The graph displays a positive correlation with a correlation coefficient of 0.771. The fitted line indicates a trend in the data. Noteworthy data points are circled.]
Beef Cows

**Proviral Load vs. Lymphocyte Count in Beef Cattle**

\[ y = 6.1135x - 10297 \]

\[ R^2 = 0.4428 \]

\[ r = 0.6654 \]
Proviral load and lymphocyte count were correlated.

BLV positive cattle (n=610)

\[ r = 0.884, \quad p = 1.2 \times 10^{-202} \]

BLV positive Holstein (n=467)

\[ r = 0.873, \quad p = 9.2 \times 10^{-147} \]

BLV positive Japanese Black (n=143)

\[ r = 0.930, \quad p = 2.3 \times 10^{-63} \]

Test Data Correlation
ELISA optical density (OD) is less correlated with lymphocyte count (LC) and proviral load (PVL)

\[ R^2 = 0.0805 \]

\[ R^2 = 0.1983 \]

*data through 12/2016*
MSU Super-shedder study

• Three herds identified for semi-annual BLV testing:
  – ELISA (milk)
  – Lymphocyte count (on ELISA positives)
  – Proviral load (on ELISA positives)
• Used this data to direct culling and segregation decisions.
  – Results are sorted by PVL and lymphocyte count
  – Culling plans are made for the most infectious/affected cattle
    • Lymphocyte count – our best measure of immune dysfunction
    • PVL – our best measure of infectivity for herd mates.
Preliminary Results of trial to reduce BLV transmission by selective culling or segregating cows with high proviral load and/or high lymphocyte counts

Herd (blue) that most aggressively culled high lymphocyte count and high proviral load cows saw a reduction in prevalence from 64% to 30% within the first year, while the orange herd K reduced prevalence from 58% to 44%
The decrease in prevalence in the 3 herds together was significant at $p < .0000001$ by the extended Mantel-Haenszel chi-square test for trend.

Herd H (green) is small (16% of the study cows) with no ability to segregate infected cattle.

There were only a few new cases in the milking herd, but an influx of infected heifers has prevented the overall herd prevalence from decreasing.
Direct contact

- Direct contact may be extremely important
  - Maybe why removing the super-shedders seems to work so well.

- Japanese study
  - 6 tie stall herds
  - Uninfected cattle tested every 4-6 months
  - Hazard ratio = 12.4 if tie stall “neighbor” was ELISA positive
  - What does it mean?
    - Biting flies?
    - Direct contact transmission?

Sota Kobbayashi, et al. Role of Neighboring infected cattle in BLV transmission risk. 2015. J of vet med sci
Portals of exist for BLV

- PVL was found in saliva and nasal secretions, but in much lower levels than in the blood.
- We found PVL in smegma and in one semen sample.
- Has also been found in feces and milk.
Summary: What management actions could be included in a comprehensive BLV Control Program?

- Single-use hypodermic needles  
  Field trial results discouraging.
- Single-use reproductive sleeves  
  Field trial results discouraging.
- Use AI instead of natural breeding  
  Beef natural breeding trial in fall
- No gouge dehorning. Use good medical hygiene practices for tattoos, tail cropping, ear tagging, etc.
- Control biting flies  
  Will compare seasonal rates of new infections
- Colostrum freezing or pasteurizing
- Culling or segregating ELISA positive cattle  
  Showed it works in U.S. too
- Only add purchased animals that are BLV-negative
- Genetic selection for resistance?  
  Study in progress
  – class II DRB3 gene (BoLA)
- Cull (or segregate) super-shedders  
  Field trial results encouraging

The super-shedder would seem to be the critical control point for all routes of transmission.
Bovine Leukemia Virus in Dairy Cattle:

#1 What is it?
#2 Why does it matter?
#3 What Options for control?
#4 Where to start?
What can USAHA do?

Dr. Paul Bartlett, MPH, DVM, PhD, ACVPM-Epidemiology, Professor. College of Veterinary Medicine, Michigan State University, E. Lansing, MI USA

This material is based on work supported by the United States Department of Agriculture and the National Institute of Food and Agriculture award numbers 2014-67015-21632 and 2014-68004-21881.
Where to start?

• Conduct a herd profile test
   – A BLV ELISA test on the milk from 40 cows
• If your herd has a low prevalence, you may want to consider segregating or culling the positive cows to eradicate the infection from your farm.
• If your herd has a high prevalence, you may want to reduce your prevalence with management changes before attempting eradication.
Milk samples collected by DHI for component testing (Fat, Protein, etc.) are “marked” to also be tested for BLV by the milk ELISA test.

Or collect serum samples and submit to a vet diagnostic lab.

A Herd BLV Profile tests the 10 most recently fresh cows from each of four lactation groups:

- 10 cows from lactation 1
- 10 cows from lactation 2
- 10 cows from lactation 3
- 10 cows from lactation ≥ 4

A BLV herd profile is calculated as the simple average of the percent of BLV-positive cows within each of the four lactation groups.
Herd BLV Profile

• The BLV Profile is independent of the age distribution of the herd, so comparisons can be fairly made across herds and within the same herd to past and future time periods.

• Yet – the BLV Profile is almost perfectly correlated (r=.99) with the prevalence you would obtain by testing every cow in the herd.

• The 10 most recently-fresh 1\textsuperscript{st} lactation cows usually represent transmission that occurred before entering the milking herd.
BLV Herd Profile

Milk samples collected by DHI

- The 10 most recently fresh cows from each of four lactation groups (1, 2, 3 and 4+)

BLV Extension Project Durst
1. BLV profile
2. Management change
3. Re-test in ~ 1 yr

- 2015 – 37 Michigan herds
- 2016 – 38 Michigan herds
Interpreting a BLV Profile

This pattern suggests that cattle are obtaining new infections after they enter the milking herd. Focus control on stopping transmission in the milking herd.

This pattern suggests that cattle enter the milking herd already infected. Focus control on preventing infection of calves, e.g. freezing or pasteurizing colostrum.
In summary:

• Consider conducting a BLV Herd Profile as a first step to determine your BLV status.

• Select the most appropriate approach to control BLV transmission in your herd, based on:
  – Your overall BLV prevalence
  – Whether you are targeting transmission in the young stock or in the milking herd
  – Your ability to cull or segregate positive cattle
Beef Cow BLV Longevity

Objective 1: To determine if BLV infection on cow-calf beef herds is associated with cow longevity.

3,325 samples have been collected from cows on 28 beef cattle herds in MI, OH, IN, IL, IA, MT.

Dr. Dan Grooms
Dr. Oscar Javier Benitez Rojas
Dr. Paul Bartlett

USDA NIFA 2015-67028-23652
3,325 samples have been collected from cows on 28 beef cattle herds in MI, OH, IN, IL, IA, MT. **One more year** until we do survival analysis.

### 40% positive

**Prevalence in Beef Herds Tested**

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<th>Farm Code</th>
<th>Farm Name</th>
<th>Positive</th>
<th>Negative</th>
<th>Suspect</th>
<th>Total</th>
<th>Prevalence</th>
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<th>24 month data</th>
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**Dr. Oscar Javier Benitez Rojas**
Lymphocytosis among ELISA-positive animals
A different study: Breeding soundness exams, including BLV ELISA testing, was performed on 363 Michigan beef bulls from 113 owners in the spring of 2009 (156 bulls) and 2012 (207 bulls).

- **Beef Bulls**
  
  \[ y = 12.594x - 9.9947 \]

- **Dairy Cows**

  \[ y = 7.397x + 3.008 \]
BLV prevalence is high in breeding beef bulls using for natural breeding

Dr. Oscar Javier Benitez Rojas
BLV Proviral DNA in Smegma of Seropositive Animals

- Proviral DNA was identified in the smegma of 7.4% BLV seropositive bulls (4/54)
- No PVL was found in semen.

Dr. Oscar Javier Benitez Rojas, Michigan State University
Serial sampling on bull 427

<table>
<thead>
<tr>
<th>ANIMAL ID</th>
<th>COLECT DATE</th>
<th>Lymphocyte Count</th>
<th>PVL</th>
<th>PVL SEMEN AND SMEGMA</th>
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Dr. Oscar Javier Benitez Rojas, Michigan State University
Coming attractions

• Natural breeding challenge study — Benitez Rojas
• Challenge study – natural course of infection – Hutchinson
• Deer lymph nodes and “meat juice” ELISA – Hutchinson
• Super-shedder test and cull with only lymphocyte count and ELISA - ????
Bovine Leukemia Virus in Dairy Cattle:
What is it?
Why does it matter?
What Options for control?
Where to start?
#5 What can USAHA do?

Dr. Paul Bartlett, MPH, DVM, PhD, ACVPM-Epidemiology, Professor. College of Veterinary Medicine, Michigan State University, E. Lansing, MI USA

This material is based on work supported by the United States Department of Agriculture and the National Institute of Food and Agriculture award numbers 2014-67015-21632 and 2014-68004-21881.
What should the USAHA and USDA do about BLV?

• Reasons **against** any USDA control program for BLV
  – There currently is little industry support for a national control program.
  – Our high U.S. prevalence makes the ELISA ‘test and cull’ strategy too costly for most herds.
  – Negative herds appear able to stay negative even if their neighbors are positive. So individual producers can eradicate if they wish.
  – The ELISA test for antibodies may be insufficient to the task
    • It only identifies previous infection rather than those cows that are the greatest infectious threat to their herdmates.

• Perhaps the dormant USAHA BLV-free certification program needs to be rejuvenated.
Certification of BLV-free herds

• "Standards for Certification of Cattle Herds as Bovine Leukosis Virus Free" was published by the Bovine Retrovirus Committee of the United States Animal Health Association (Miller and Lyle, 1998).”

• “This is a voluntary certification program that requires that producers obtain the services of accredited veterinarians to collect and submit laboratory specimens for analysis using a diagnostic test that has been approved by USDA Animal and Plant Health Inspection Service.”

• I have been unable to locate any other information about this USAHA program from 1998.  http://www.cabi.org/isc/datasheet/91714

STANDARDS FOR CERTIFICATION OF CATTLE HERDS AS BOVINE LEUKOSIS VIRUS FREE

I. Introduction
Owners of cattle participating in the voluntary certification program are required to obtain the services of accredited veterinarians and to submit samples to the National Veterinary Services Laboratories or other laboratories approved by the National Veterinary Services Laboratories to conduct tests for bovine leukosis. The serologic test(s) to be used must be approved by USDA, APHIS.

II. Definitions
A. Herd
1. All cattle under common ownership or supervision that are grouped on one or more parts of a single premises (lot, farm, or ranch). More than one herd may be maintained on a single premises if they are separated to preclude any physical contact between herds and have separate feed, water and drainage systems, or
2. All cattle under common ownership or supervision on two or more premises that are geographically separated, but on which cattle have been interchanged or where there has been contact among

BLV Certification Plan

There have been some developments since 1998

• Need allowances for ELISA milk testing
• Most testing these days is DHI-collected milk samples
  – Not sure if these labs are “accredited” by USDA
• The recertification plan should make allowances for:
  – Bulk tank screening
  – Pooling of samples (perhaps 20 milk samples pooled)
Examples of other USDA Certification Programs

• Scrapie Free Flock Certification Program

• USDA Herd Certification Program for Chronic Wasting Disease in the U.S.

• Uniform Program Standards for the Voluntary Bovine Johne’s Disease Control Program

• USDA Approved Organic

• UNIFORM METHODS FOR THE ESTABLISHMENT AND MAINTENANCE OF BOVINE LEUKOSIS VIRUS-FREE HERDS BY THE MISSOURI DEPARTMENT OF AGRICULTURE
For references and more information, please see our website at WWW.BLVUSA.com

This material is based on work supported by the United States Department of Agriculture and the National Institute of Food and Agriculture award numbers 2014-67015-21632 and 2014-68004-21881.
Thank you!

Check out our website:  WWW.BLVUSA.com