CentralStar

What's New on the Horizon: CentralStar PCR Diagnostics

Kelly R.B. Sporer, PhD

Research Scientist/Diagnostic Technical Specialist

Kelly.Sporer@mycentralstar.com 517-908-0500

CentralStar

Our Team

Research and Development (Lansing & Grand Ledge, MI)

Director: **Dr. Todd Byrem**

Associate Director: **Dr. Casey Droscha**

Research Scientist/Diagnostic Technical Specialist: **Dr. Kelly Sporer**

DVM/MS Student: **Katy Kesler**

2 Research Associates: **Kelsey Brigham & Chaelynne Lohr**

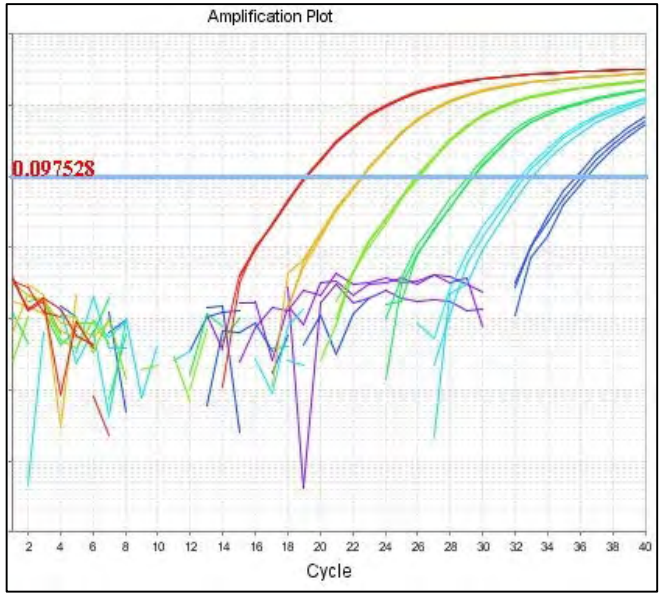
R&D intern: **Jessie Zenchak**

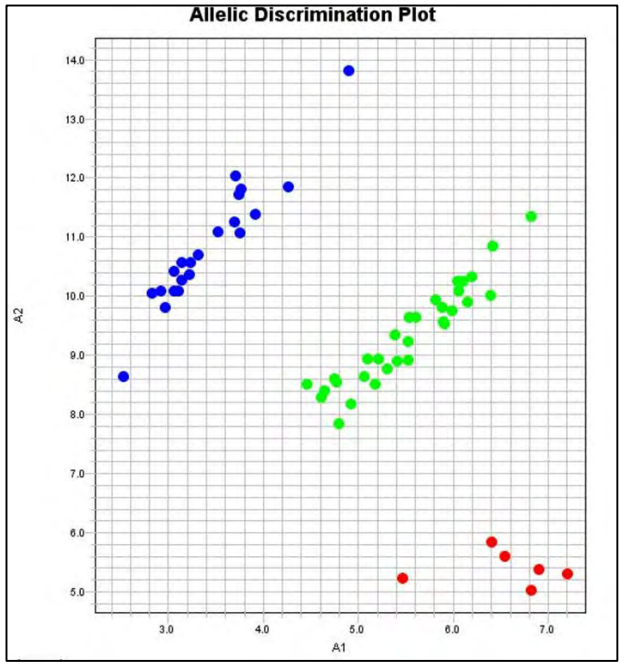
MI Lab (Grand Ledge): 4 diagnostic technicians; 1 summer intern

WI Lab (Kaukauna): 3 diagnostic technicians

Automated Nucleic Acid Extraction

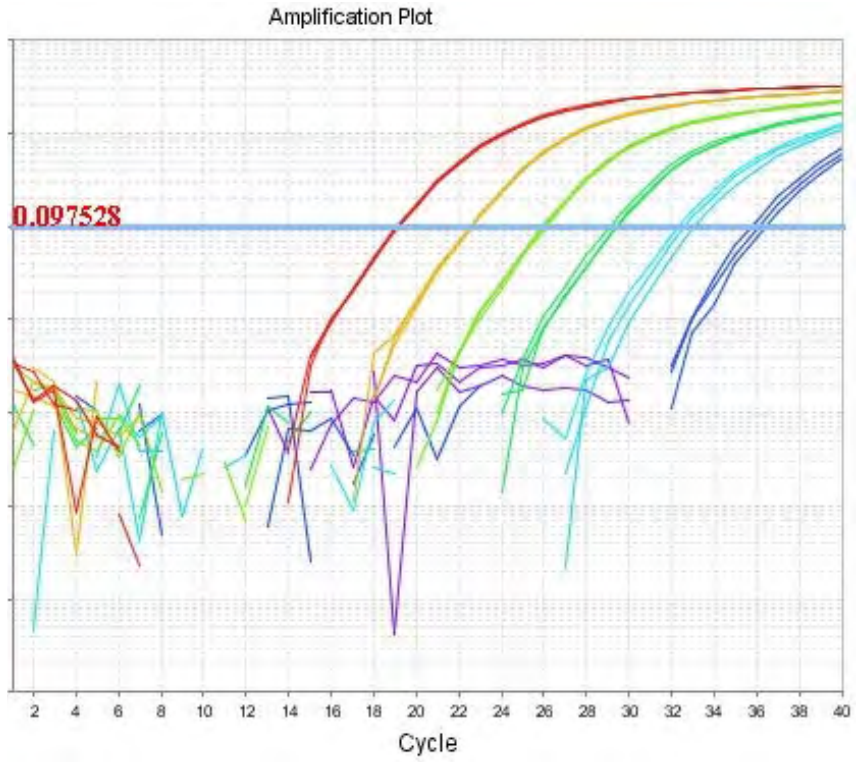
- Fecal samples
- Whole Blood
- Tissue (ear notch)
- Milk





Emerging Diagnostics

- Leukosis (BLV) PCR: SS1 test
- β-casein (A1/A2) genotyping

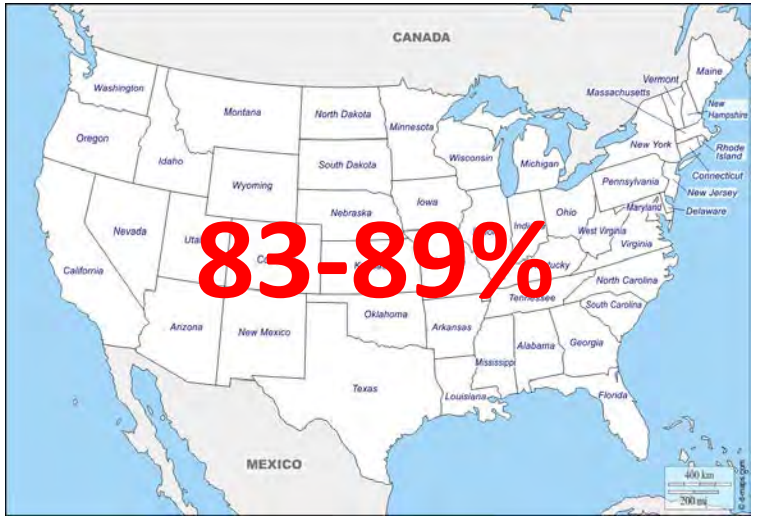


BLV PCR (SS1 assay)

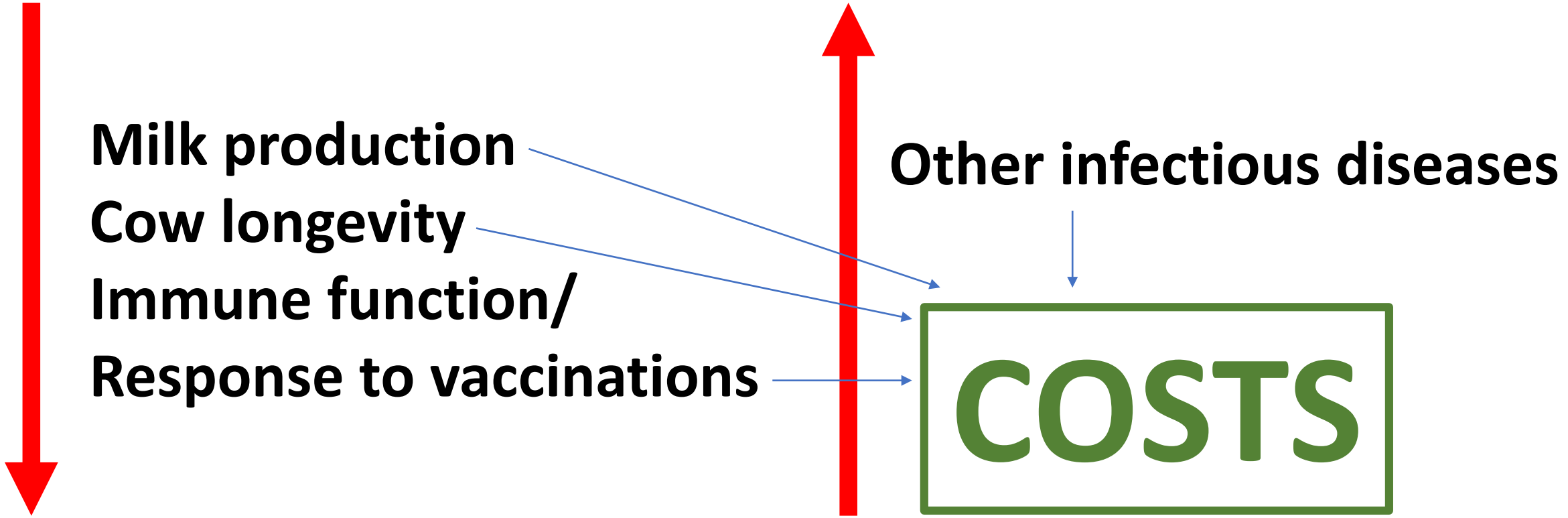
Bovine leukosis

Bovine Leukemia Virus

- Infects white blood cells of cattle/incorporates its own DNA
- Can cause abnormal cell growth/lymphoma
- Most cows asymptomatic
- Eradicated in 22 countries



Why should we care about BLV?

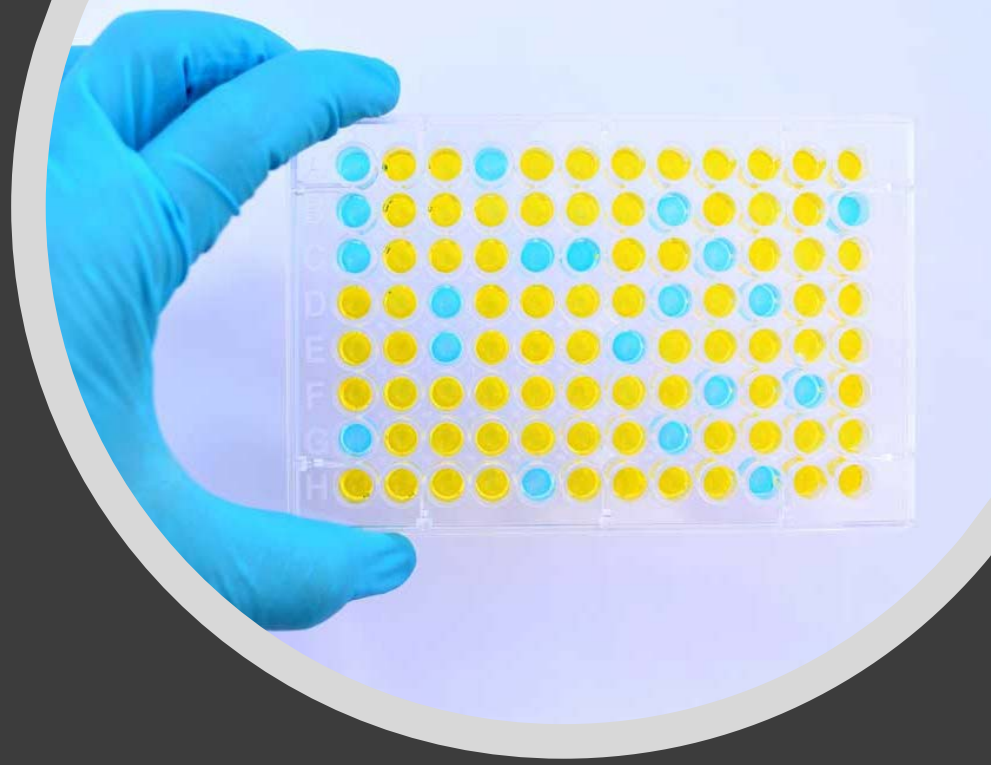


Erskine et al., 2011; Bartlett et al., 2013; Norby et al., 2015; Frie et al., 2017

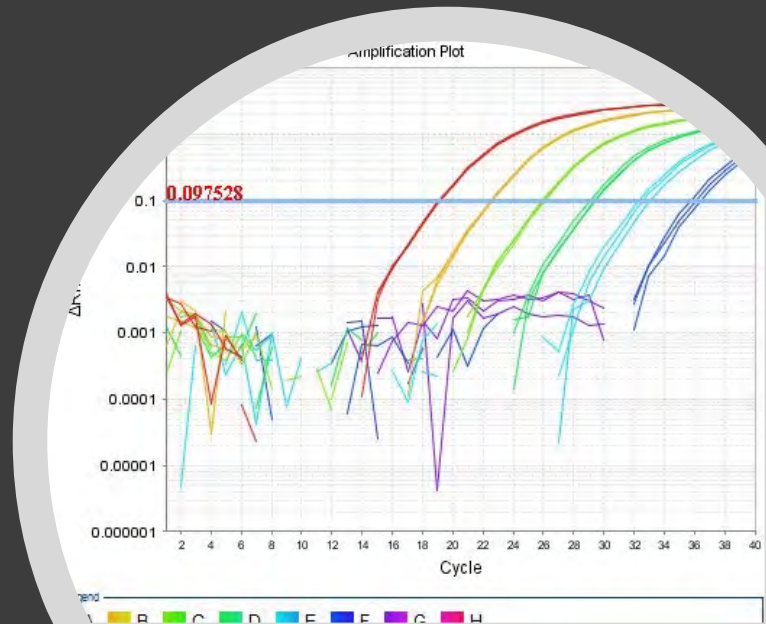
How is BLV spread?

How can we diagnose BLV?

- **ELISA = Enzyme-linked ImmunoSorbent Assay**
  - Measures cow's immune response to virus
  - Measured in blood (serum or plasma) or milk
  - Relatively cheap

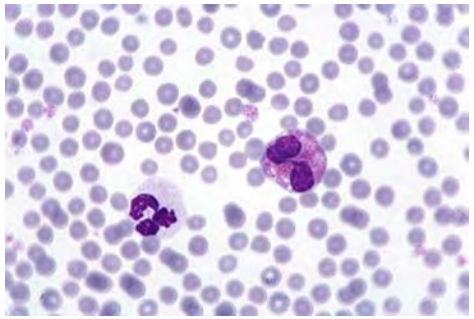


- **PCR = Polymerase Chain Reaction**
  - DNA test: measures copies of virus (proviral load or PVL)
  - Measured in whole blood
  - More sample processing; expensive



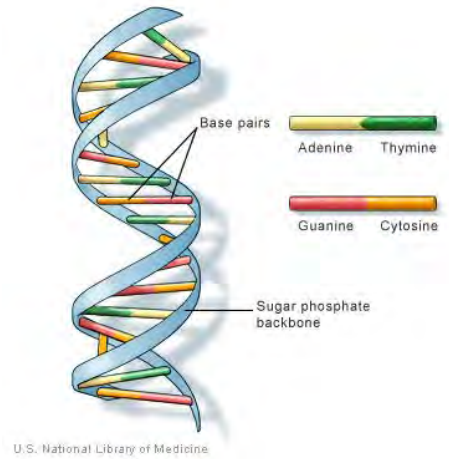
How can we diagnose leukosis?

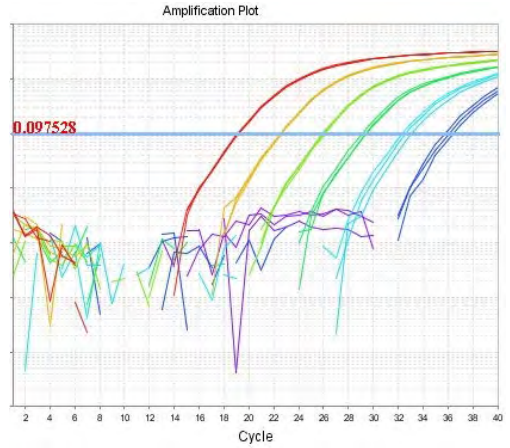
- **White Blood Cell Counts**
  - Costs vary
  - Specificity?



What is CentralStar doing about BLV?

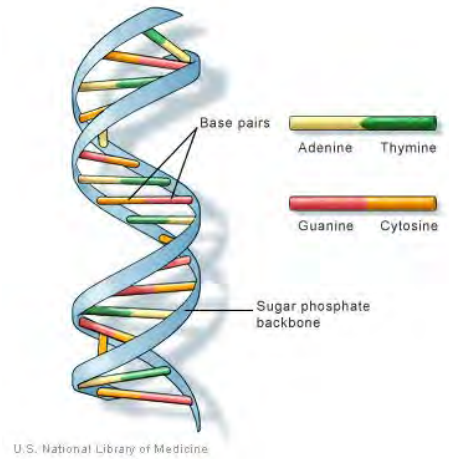
- Helping herds determine/reduce their prevalence
- Investigating when cows get infected/when they become most infectious
- Developing better diagnostic tests to find the most contagious cows (“Super-Shedders”)
- Identifying genetic markers for resistance/susceptibility

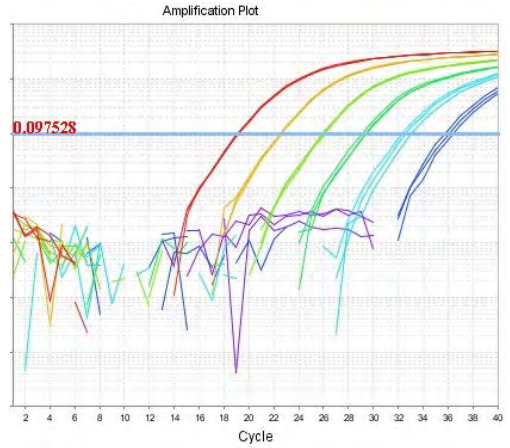




What is CentralStar doing about BLV?

- Helping herds determine/reduce their prevalence
- Investigating when cows get infected/when they become most infectious
- Developing better diagnostic tests to find the most contagious cows (“Super-Shedders”)
- Identifying genetic markers for resistance/susceptibility





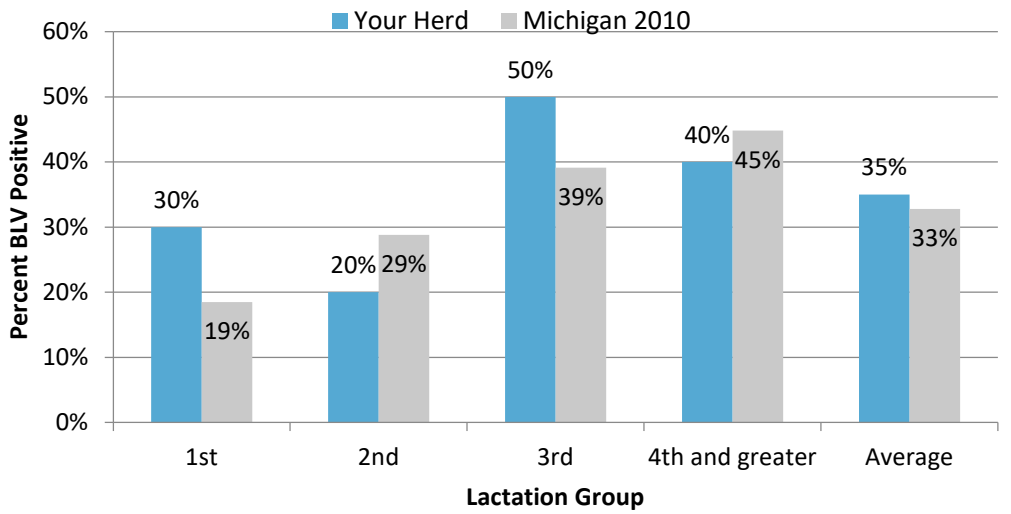
Helping herds determine their prevalence

Current Action:	Frequency:		
	Never or Rarely	Sometimes	Frequently or Always
1. Use needles only once (new needle for each heifer and cow)		✓	
2. Use palpation and A.I. sleeves only once (new sleeve for each heifer and cow)		✓	
3. Use natural service bulls	✓		
4. Use some type of fly control			✓
5. Pasteurize or freeze colostrum			✓
6. Feed pooled, unpasteurized waste milk to calves	✓		
7. Segregate BLV-positive animals	✓		
8. Sanitize equipment used on cattle between animals (scoop or gouge dehorners, tail docking, tattoo pliers, balling gun, etc.)			✓

Helping herds determine their prevalence

n = 10 freshest from lactations 1, 2, 3, and 4+
40 cows total tested by BLV milk ELISA at no charge

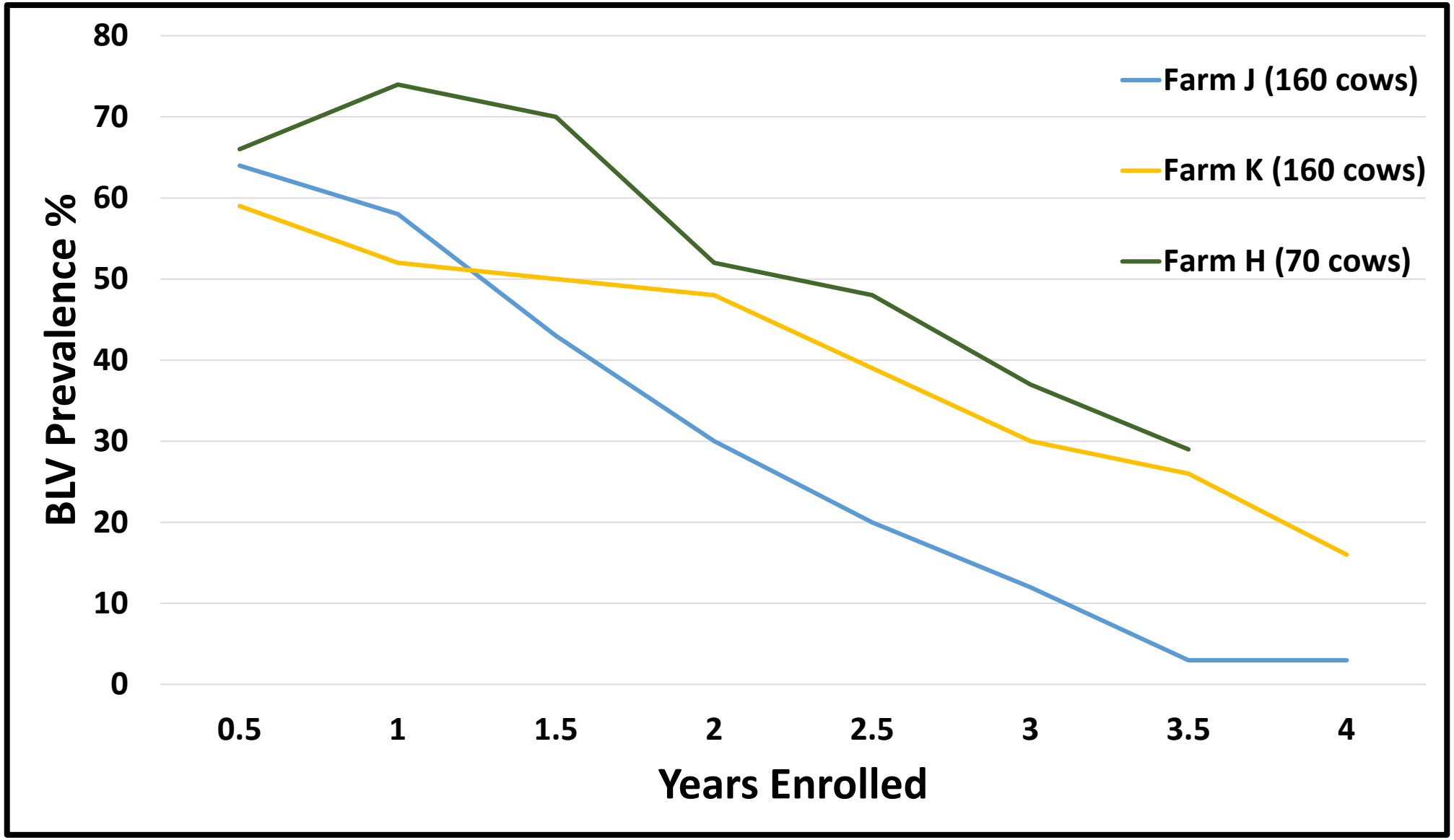
Erskine et. al, 2012



Expected RHA Improvement (lbs) with BLV Profile Improvement

		Target BLV Profile							
Number Positive	Current BLV Profile	87	75	62	50	37	25	12	0
		RHA improvement (lbs)							
40	100	290	615	896	1,210	1,500	1,815	2,105	2,420
35	87		290	615	896	1,210	1,500	1,815	2,105
30	75			290	615	896	1,210	1,500	1,815
25	62				290	615	896	1,210	1,500
20	50					290	615	896	1,210
15	37						290	615	896
10	25							290	615
5	12								290
0	0								

Identifying and removing “Super-shedders” significantly decreases BLV herd prevalence



HOARD'S DAIRYMAN

Stop BLV super-shedders in their tracks

by Paul Bartlett, Vickie Ruggiero, Philip Durst, Casey Droscha, Kelly Sporer, and Todd Byrem

COWS THAT DON'T APPEAR SICK may still be carries of bovine leukemia virus (BLV). Finding and removing super-shedders appears to be a critical control point to reduce BLV transmission within the herd.

Abby Bauer

THE prevalence of bovine leukemia virus (BLV) in U.S. dairy cattle was about 10 percent in the 1970s. Since then, that number has slowly climbed to approximately 45 percent of all dairy cattle infected, while about 95 percent of herds have at least one positive cow. Lymphoma tumors induced by BLV are the leading cause of cattle condemnation.

We are now realizing that even greater than previously suspected economic losses are associated with the reduced milk production and shortened cow lifespan that come with BLV. Many recent studies have discovered that BLV disrupts the cow's immune system in multiple ways. This immune disruption is thought to our recent national survey of 103 dairy producers indicated that only about 10 percent viewed BLV as a significant problem.

If your herd's BLV prevalence is typical (about 45 percent), it would be economically difficult to cull all your BLV ELISA-positive cows, as well as a normally lower percentage of your young stock. So, how can you reduce your prevalence down to 5 to 10 percent where culling all the infected cattle could be feasible to eradicate the disease from your herd?

Our Michigan State University Extension study of 80 Michigan herds that tested annually for at least three years showed limits to what we can achieve by management BLV antibody ELISA test only measures the amount of antibody present and is lowly correlated with proviral load and lymphocyte count.

The proviral load can differ vastly among ELISA-positive cattle in that some cows can have several thousand times more provirus per volume of blood than other ELISA-positive herdmates. The term "super-shedder" is used for cattle with high proviral load that represent the greatest infectious threat to their herdmates.

These super-shedders are an obvious critical control point for the many routes of BLV transmission, including direct contact with nasal secretions, milk, saliva, semen, and feces. Transmission can also come through biting flies, colostrum, milk, and various types of blood borne transmission via hypodermic needles, reproductive sleeves, hoof trimming, ear tagging, and gouge dehorning. All these routes rely heavily on the presence of the super-shedders, which may be the weakest link for breaking the chain of transmission.

Our three-herd pilot field trial was the first ever demonstration that a significant reduction in BLV transmission (and prevalence) could be achieved if super-shedders were regularly removed from the herd. Every six months, these three milking herds were screened by milk ELISA through DHI.

The ELISA-positive cattle were then blood tested for proviral load (and sometimes also for lymphocyte count). The most infectious cattle were usually quickly culled, and moderately infectious cattle were separated as much as possible until they could be culled.

The decline in BLV prevalence after two and a half years is shown in the figure below. In comparison, systems and even different breeds of cattle.

The pilot study results are encouraging in suggesting that this method can reduce prevalence to a low level where it could become economically possible to eradicate BLV from the herd by culling all the remaining ELISA-positives. This has been done in thousands of herds in other nations. Inexpensive post-eradication monitoring methods to assure continued absence of BLV are well-tested in these other nations.

A test for the future

The proviral load test we had been using until spring 2018 for our research is very laborious and, therefore, quite expensive. A new proviral load test is being developed that will hopefully soon be available though the DHI organization. We have begun using this new test for our expanding field trial and for other BLV research projects, and the results are extremely encouraging.

While it may be a couple of years before the test is widely available, dairy farmers and their veterinarians can take steps now in advance of the new test. The first step we recommend is to have a BLV Herd Profile test done. This is usually done as an ELISA test of milk samples from 40 cows in the herd; the 10 most recently fresh of first, second, third, and fourth or greater lactations. Most DHI technicians are becoming familiar with this procedure.

Secondly, within-herd biosecurity should be practiced to reduce the risk of transmission of BLV among your cows by using single-use needles for all injections and single-use sleeves/gloves for rectal exams and insemination. Colostrum fed to calves should be frozen or pasteurized.

J. Dairy Sci. 102
https://doi.org/10.3168/jds.2018-16186

Controlling bovine leukemia virus in dairy herds by identifying and removing cows with the highest proviral load and lymphocyte counts

V. J. Ruggiero,[1]* B. Norby,[1] O. J. Benitez,[1] H. Hutchinson,[1] K. R. B. Sporer,[1,2] C. Droscha,[1,2] C. L. Swenson,[3] and P. C. Bartlett[1]
[1]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing 48824
[2]NorthStar Cooperative, Lansing, MI 48910
[3]Department of Pathobiology and Diagnostic Investigation and Veterinary Diagnostic Laboratory, Michigan State University, East Lansing 48824

The SS1 Field Trial

DHI Test

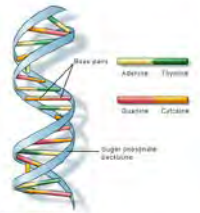
SCC and other components
Leukosis ELISA value

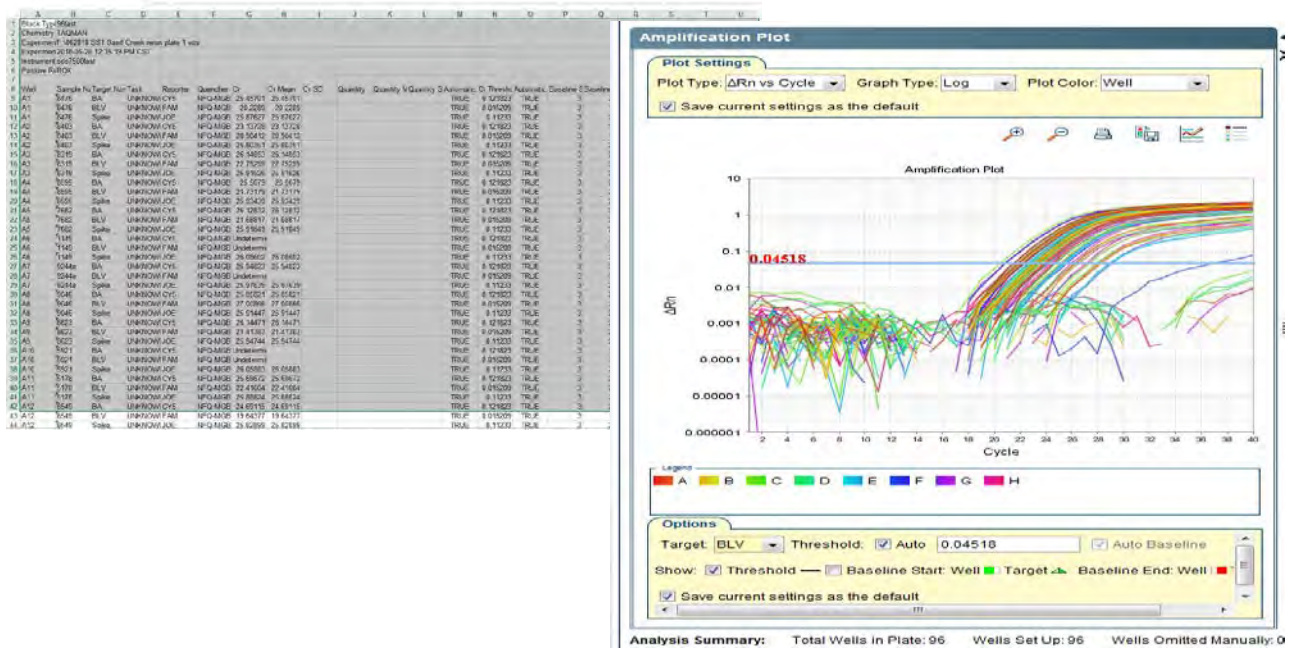
ELISA-positive cows

Blood Tube

DNA

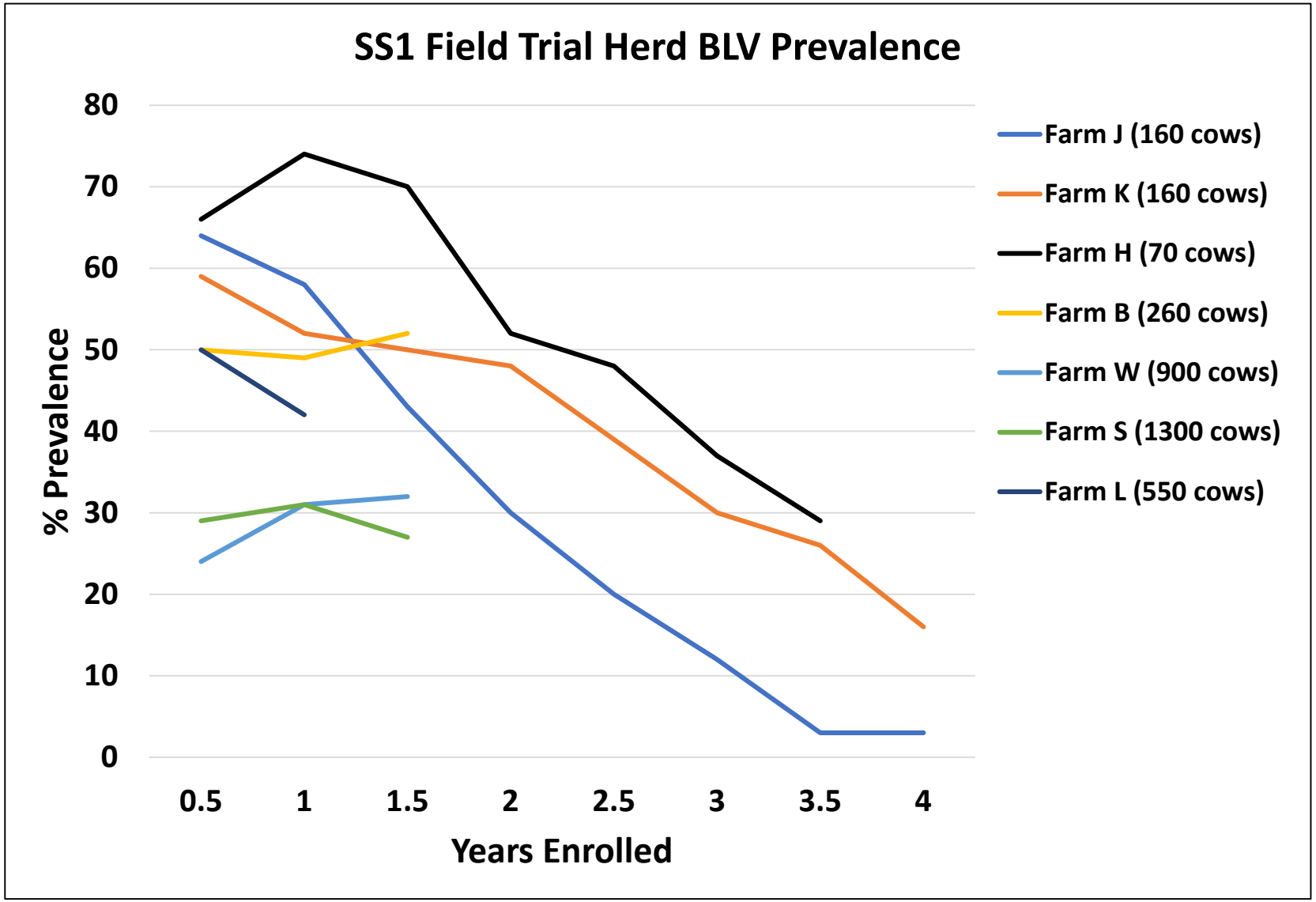
Central Star SS1 PCR test



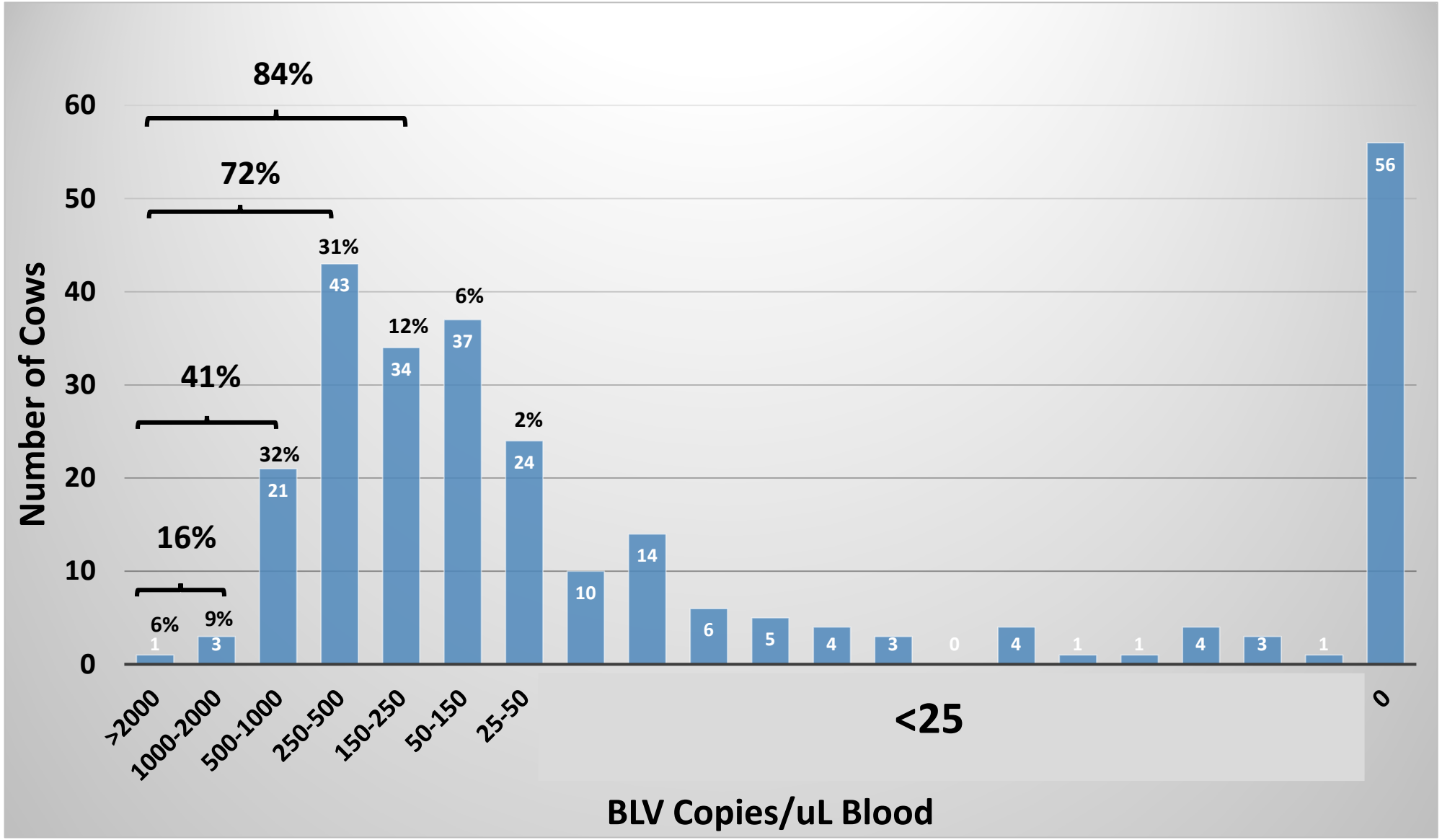


3 herds
~ 350 cows

7 herds
>3000 cows



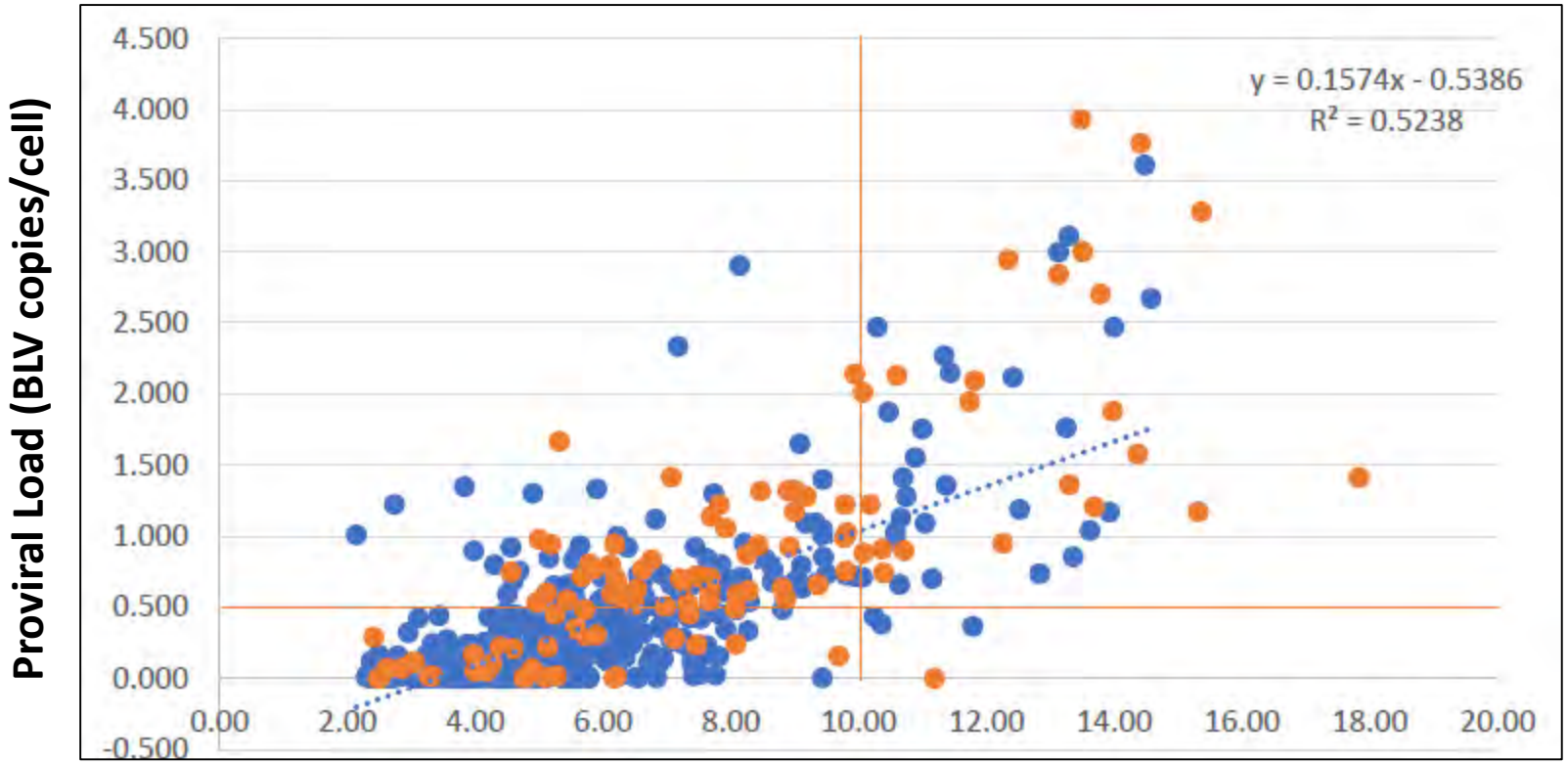
Farm S (~1100 cows) Whole Herd BLV Distribution



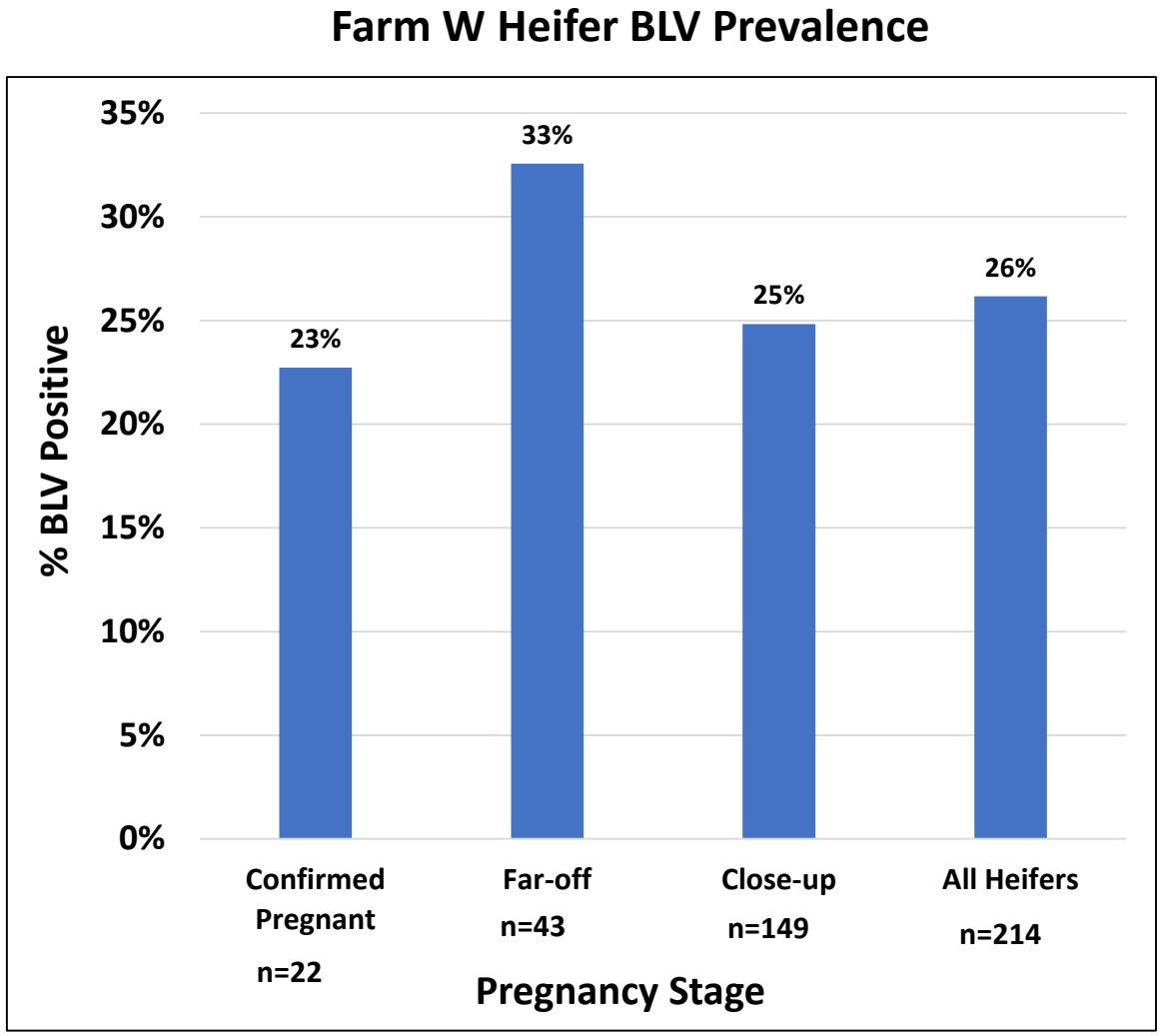
- 25 cows responsible for over 1/3 of BLV shedding in herd

Herd D: employing lymphocyte count, ELISA, SS1 PCR to eradicate leukosis

~3300 cows



New infections: role of young stock



Farm Prevalence ~32%

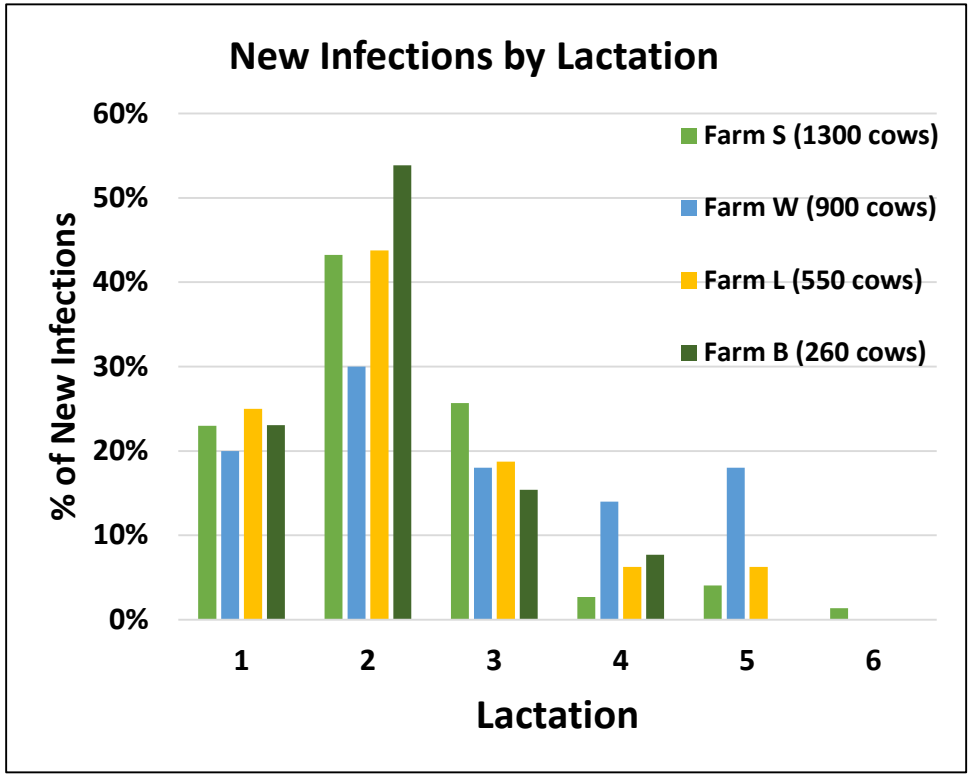
Farm K Heifer BLV Prevalence

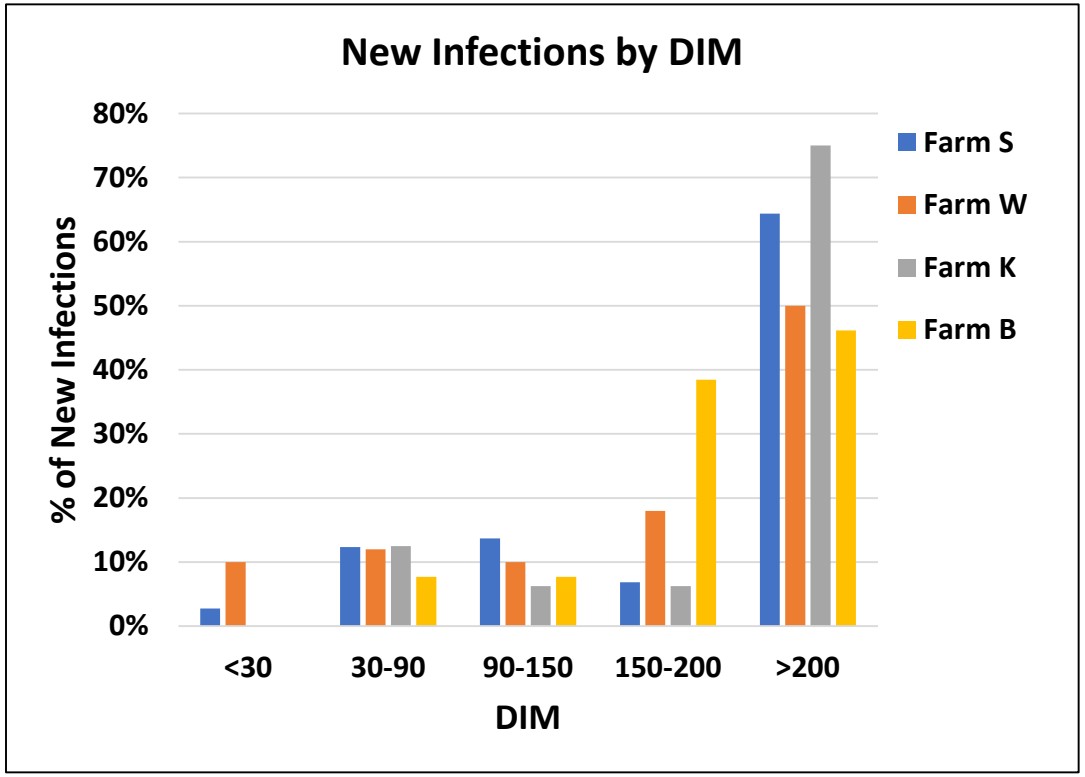
600 lbs heifers	n=10
Being bred	n=16
Confirmed pregnant 30-60d	n=10
>3 months pregnant	n=13
Springers	n=6
Total	n=54

ALL BLV NEGATIVE

Farm Prevalence ~42%

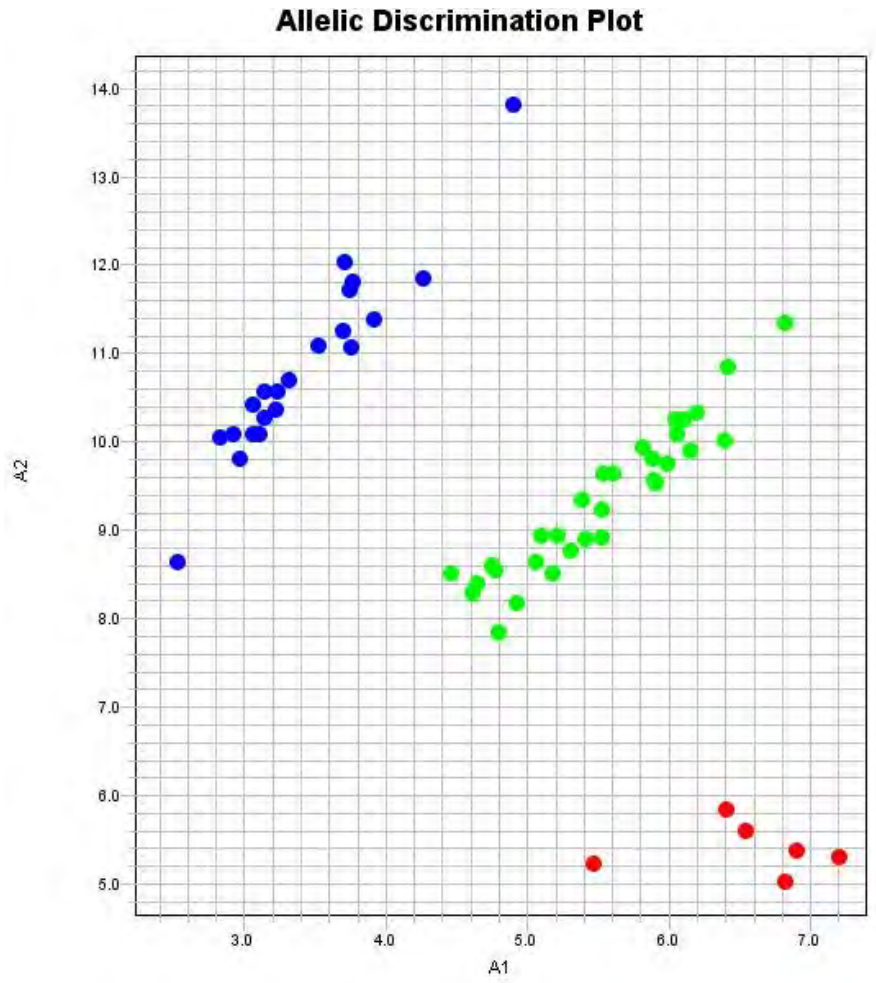
New infections: when are they happening?





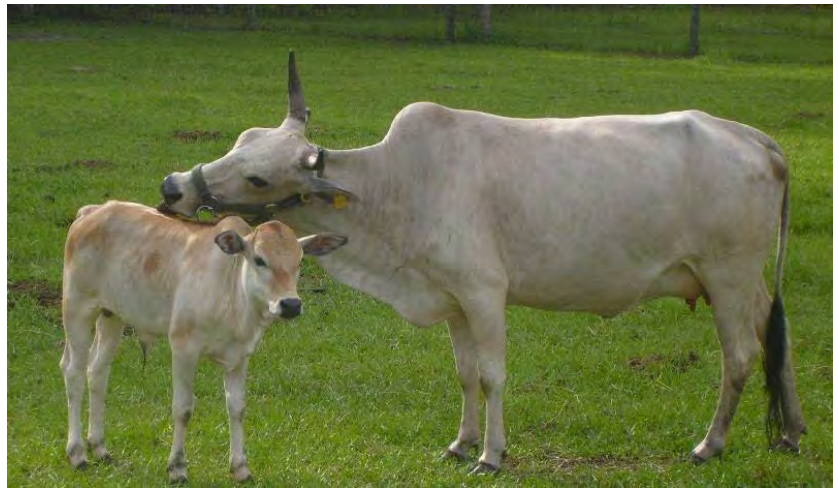
USDA NIFA AFRI grant proposal submitted 7/9/2019:

"Establishing a comprehensive approach for eradication of BLV: Reducing BLV incidence in young stock while removing super-shedders from the milking herd"



β-casein (A1/A2) genotyping

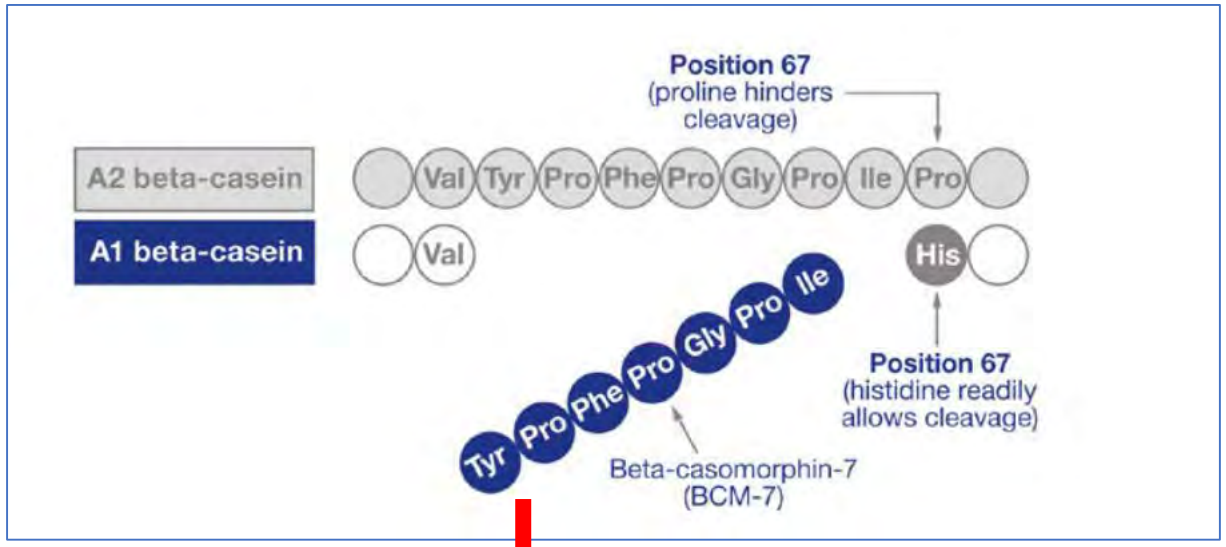
A1/A2 Testing: In Demand



Frequency of β-casein alleles

Breed	A1	A2
Guernsey	1-6%	88-97%
Jersey	9-22%	49-54%
Holstein	31-49%	49-62%
Ayrshire	72%	28%

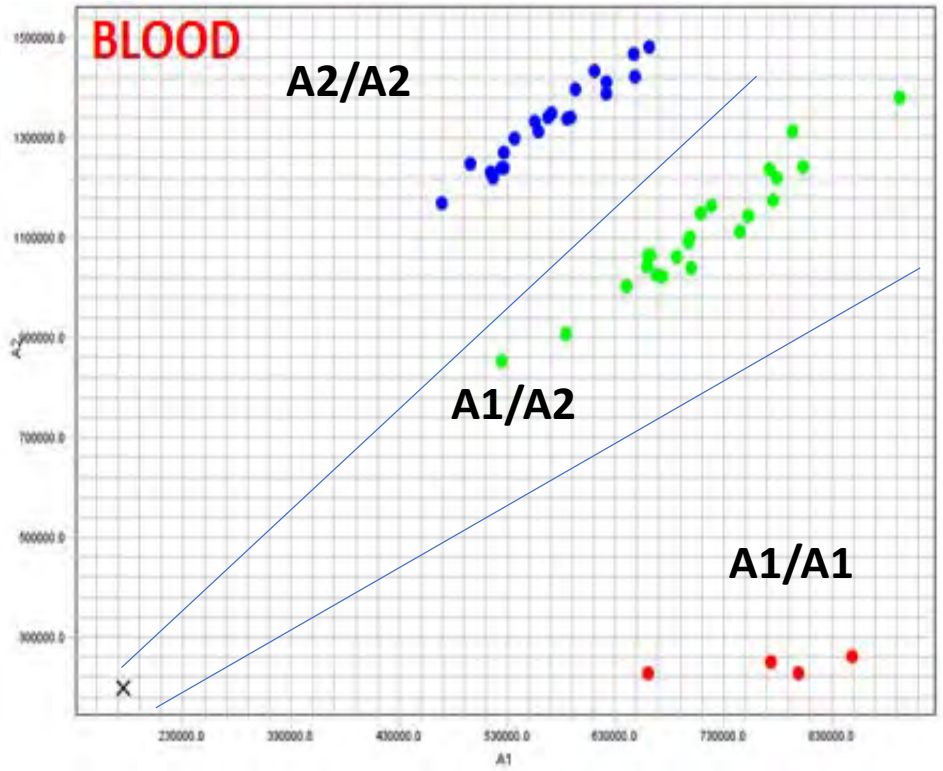
**Frequency of B allele not shown; Taken from Kaminski et al., 2007*



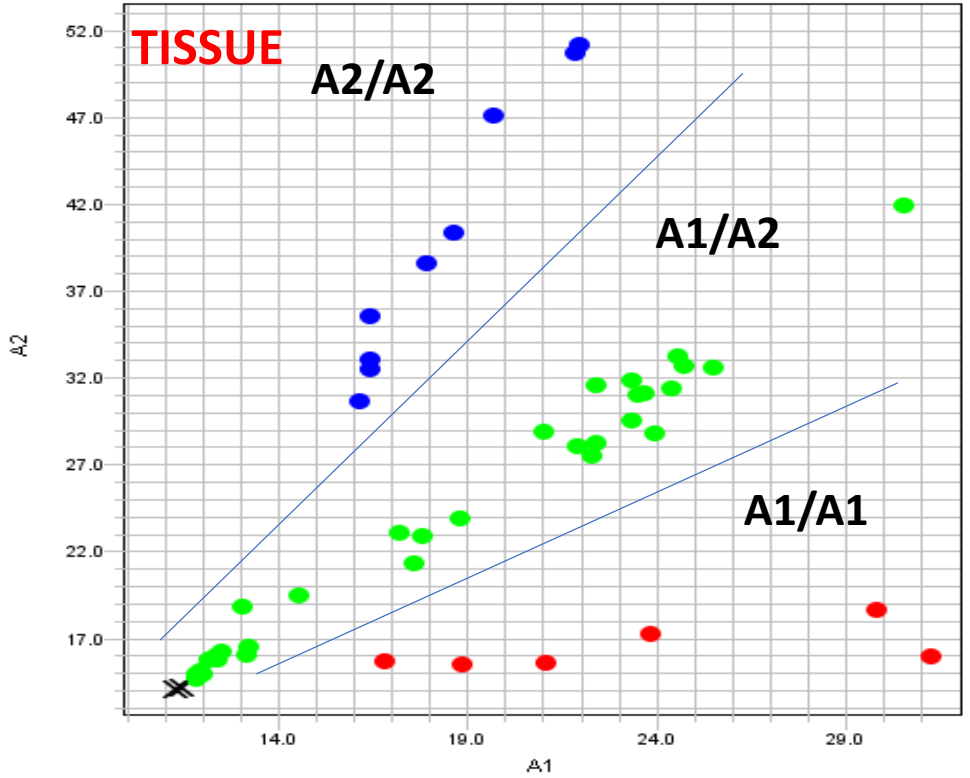
Reviewed by Pal et. al, 2015

Milk Intolerance
Ischemic Heart Disease
Diabetes mellitus
Obesity
Sudden Infant Death Syndrome

A1/A2 genotypes easily separate in blood and tissue (ear notch) using AntelBio PCR assay

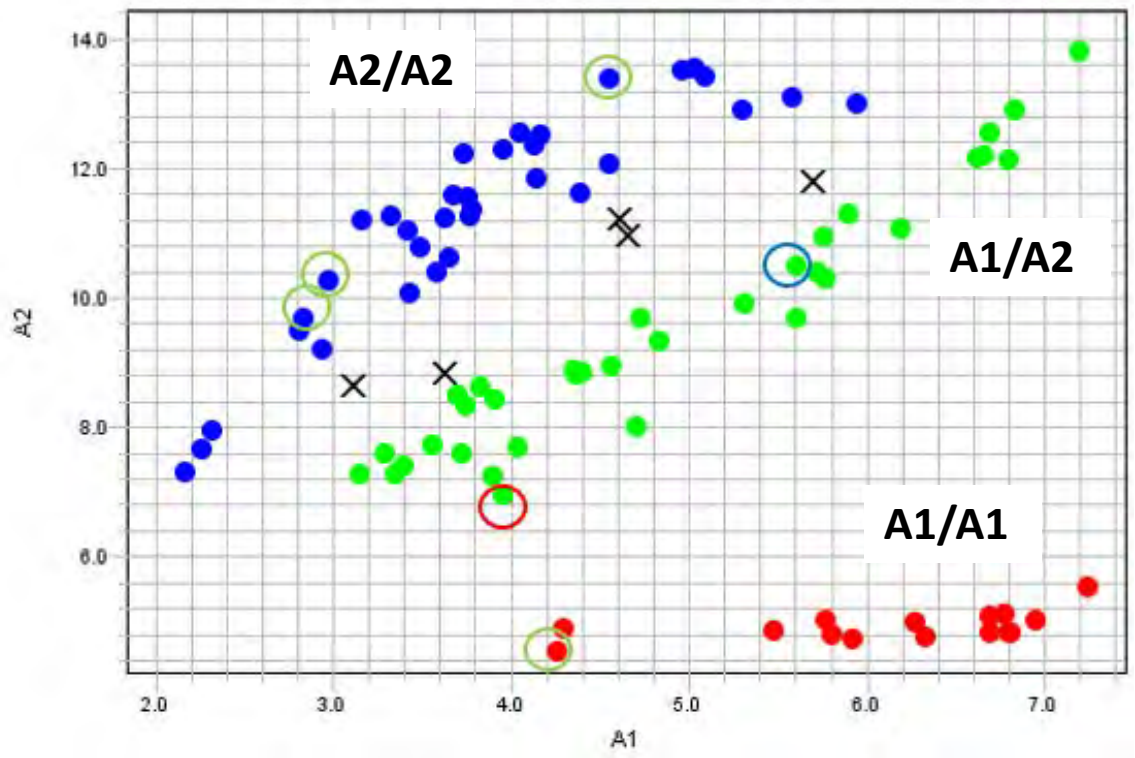


98.7% accuracy* when compared to known results

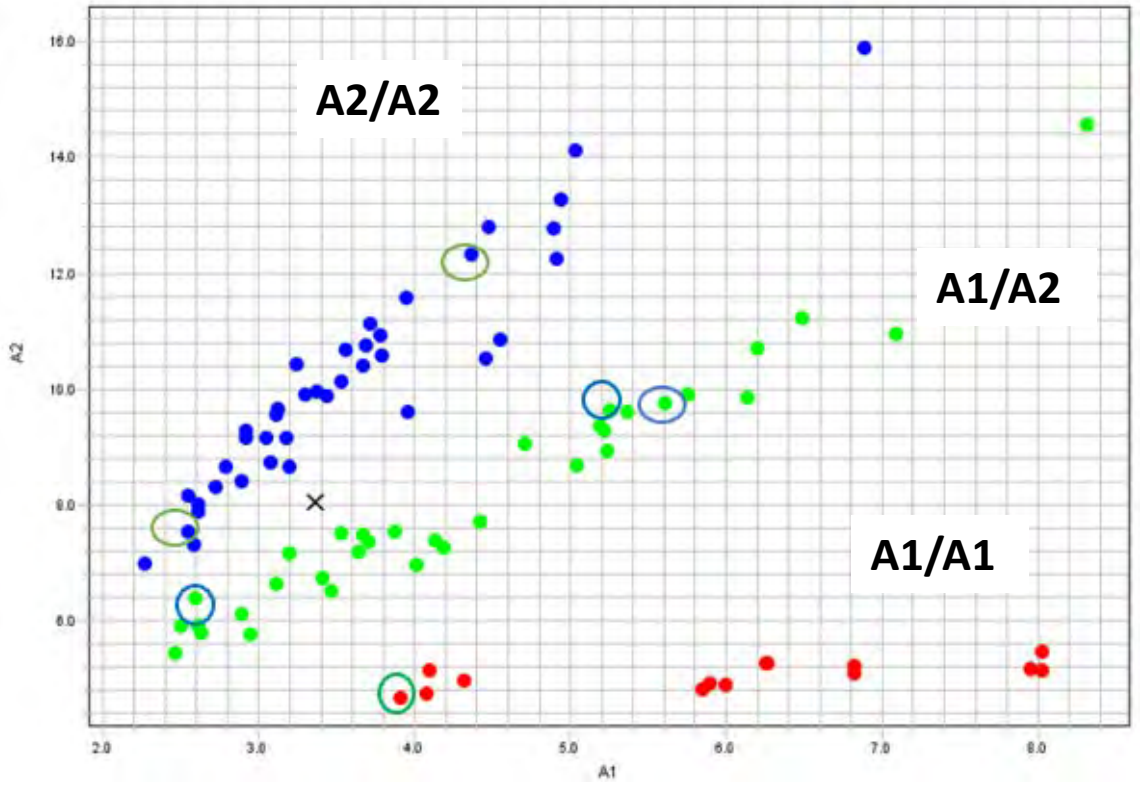


Still need to compare to “known” results

A1/A2 genotypes DO NOT easily separate in milk



DHI Milk



Hand-stripped Milk

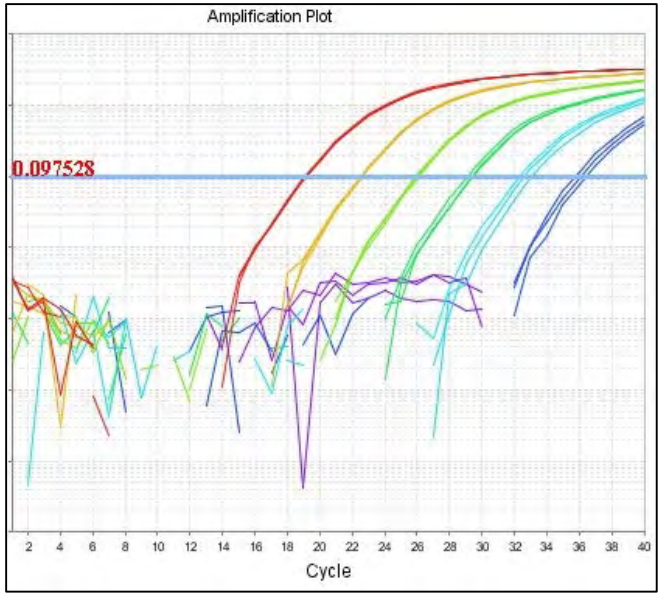
CentralStar

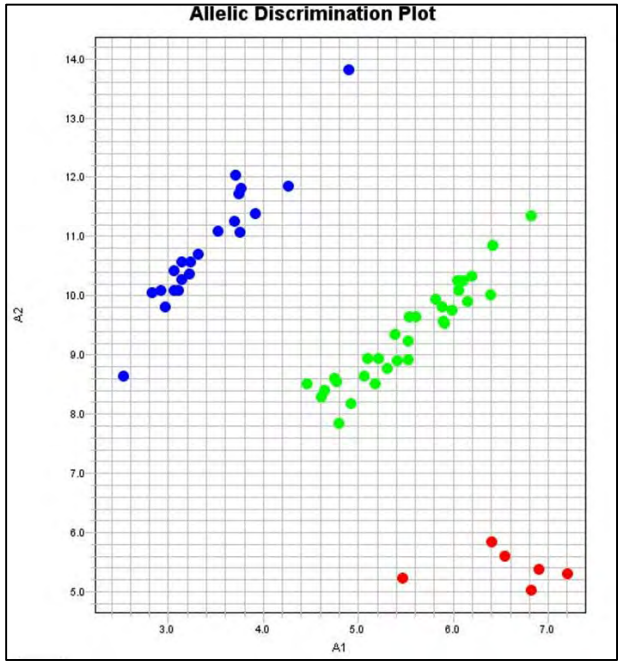
Customer Contact Info	Tested by: Kelsey Brigham, Research Associate Results date:
Customer #:	

Individual Blood A1/A2 Results

Cow ID	Barn Name	Age	A1/A2 Status
398	Brownie	3	A1/A1
399	Lisa	5	A1/A1
400	Jillian	4	A1/A1
401	Spot	6	A1/A1
381	Milkyway	2	A1/A2
389	Twinkle	2	A1/A2
387	Melissa	2	A1/A2
351	Regina	4	A1/A2
402	Sprinkles	2	A1/A2
403	Donut	3	A1/A2
404	Chocolate	5	A1/A2
405	Shania	4	A1/A2
406	Emmylou	2	A1/A2
407	Dolly	2	A1/A2
397	Carrie	2	A2/A2
408	Bonnie	3	A2/A2
409	Loretta	4	A2/A2
410	Patsy	2	A2/A2

For research purposes only.





Emerging Diagnostics

- Leukosis (BLV) PCR: SS1 test
- β-casein (A1/A2) genotyping

Inspire. Innovate. Integrate.