Haplotype inheritance and livability of recumbent Holstein calves

E. L. Nicolazzi [1], A. Al-Khudhair[2], P.M. VanRaden [2*], D.J. Null[2] , M. Neupane[2], C.D. Dechow [3]

[1] Council on Dairy Cattle Breeding (CDCB), Bowie, MD, USA

[2] USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD, USA.

[3] Pennsylvania State University.

Before we start…

Credit where credit is due

- This presentation is a multi-partner collaborative work
  - Public, academic and private collaboration
- Led by USDA Animal Genomics and Improvement Laboratory (AGIL)
- Presentation by Ahmed Al-Khudhair

Early onset muscle weakness > Calf recumbency

- Official recognition as a recessive still in progress
- Naming might not be final
- Name/acronym used (MW) is not final nor official

Background:
Newly discovered recumbency disorder (MW) in Holstein breed.

JDS Communications®
2022; 3:412–415

https://doi.org/10.3168/jdsc.2022-0224
Short Communication
Genetics

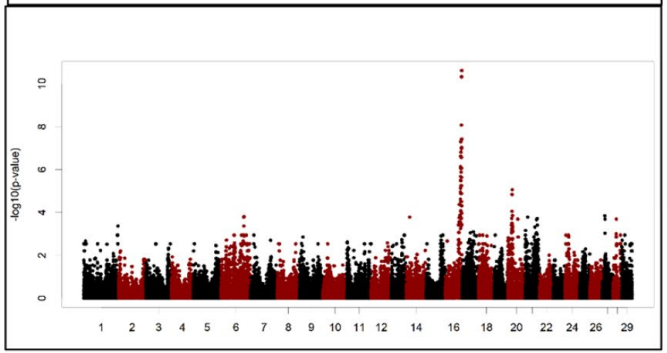
Identification of a putative haplotype associated with recumbency in Holstein calves

C. D. Dechow,[1]* E. Frye,[2] and F. P. Maunsell[3]

Identification of an incompletely penetrant haplotype on chromosome 16 that is associated with Holstein calf recumbency

Calves on multiple farms were unable to stand without assistance

The calf recumbency phenotype was associated with a haplotype on chromosome 16



https://www.ars.usda.gov/ARSUserFiles/80420530/Publications/ARR/Haplotype%20tests_ARR-Genomic5.pdf

Objectives:

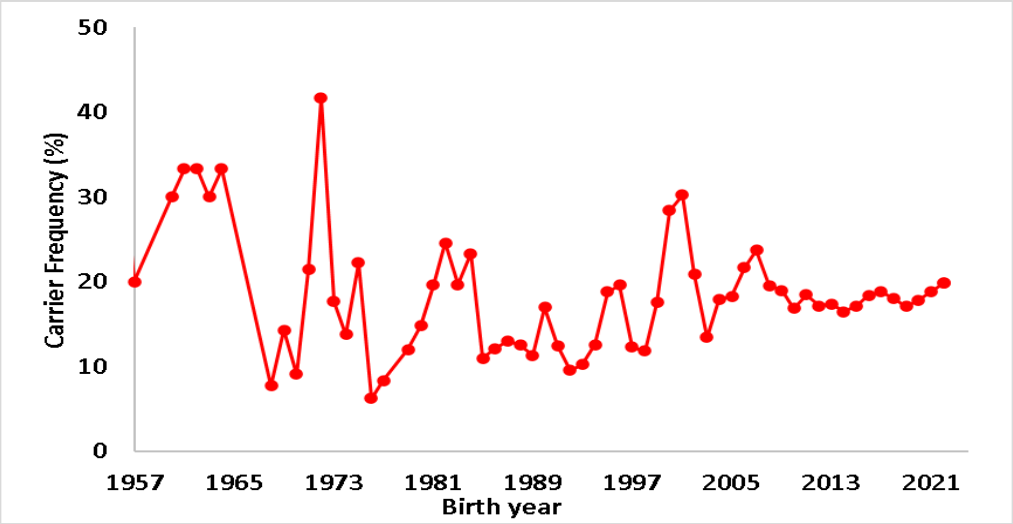
1. Investigate potential genetic association for Holstein MW
   - ✓ haplotype level.
   - ✓ Sequence level: is there a recent mutation causing and/or associated with the MW disorder?

2. Validating penetrance from heifer livability / stillbirth data and its possibility of confounding the MW phenotype results.

3. Tracking such genetic disorders by utilizing pedigree data to track potential new mutations within existing haplotypes.

(4.) Develop a routine haplotype test and compare results to (new) lab test results.

Confirming a recent mutation hypothesis in the MW haplotype (HMW)



- HMW has much less calf mortality than expected
- Hypothesis of a recent mutation in the MW haplotype (HMW)

Carrier and homozygote frequencies for the main MW haplotype without pedigree restriction.

	Females		All males		Published males	
	N	%	N	%	N	%
Total	4,931,321	100	391,960	100	285,276	100
Carrier	848,701	17.21	65,558	16.73	48,538	17.01
Homozygous	41,341	0.84	3,225	0.82	2,381	0.83
Expected homozygous	43,573	0.88	3,318	0.85	2,490	0.87

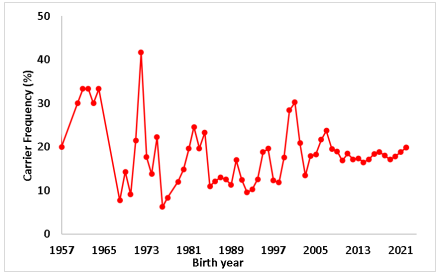
Confirming a recent mutation hypothesis in the MW haplotype (HMW)

Sequence variants with the highest concordance associated with the recumbency disorder (ARS-UCD1.2/bosTau9, region of (75,761,625 - end of chr16)).

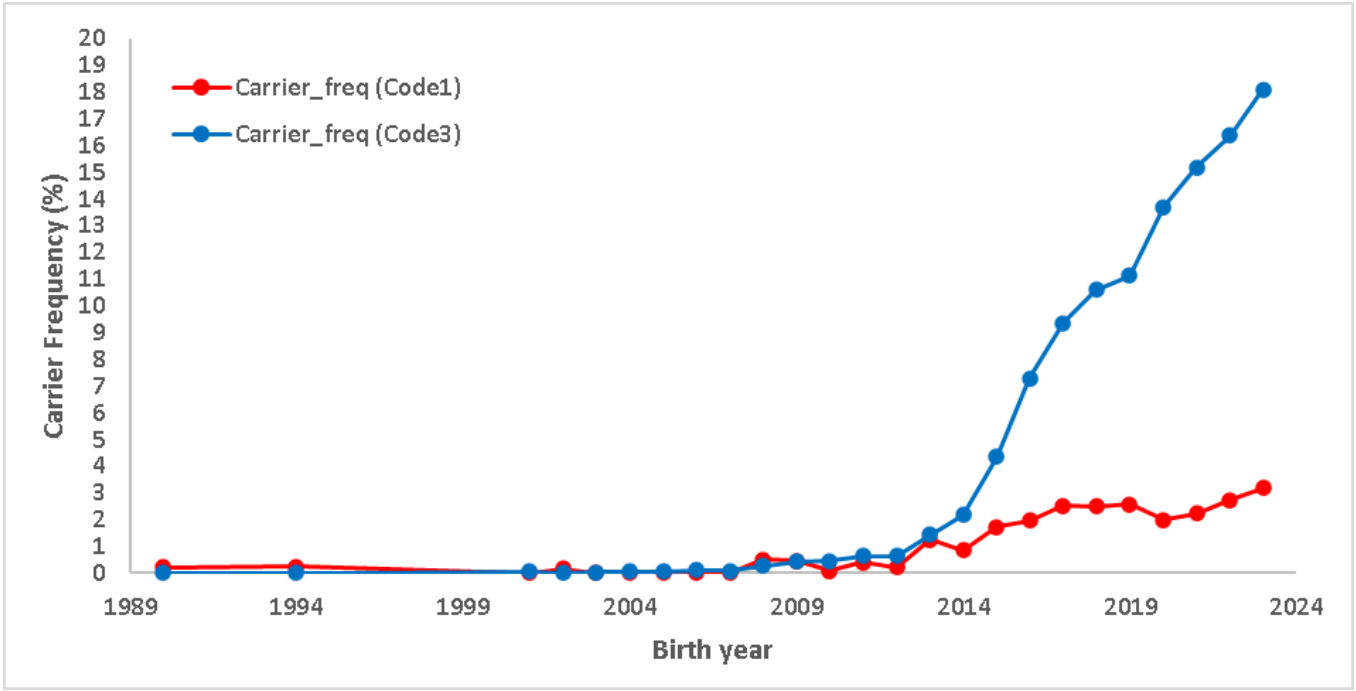
Location	Type	Priority	Gene	Match	No match	G missing	Call total	Call rate %	Concordance %
79246884	synonymous	low	KIF14	81	2	216	83	28	98
79636921	missense/splice region	moderate	CACNA1S	151	4	144	155	52	97
79733060	synonymous	low	ENSBTAG00000046177	213	7	79	220	74	97
79613592	**missense**	**moderate**	**CACNA1S**	**256**	**9**	**34**	**265**	**89**	**97**
79733068	missense	moderate	ENSBTAG00000046177	227	8	64	235	79	97
76107580	missense	moderate	ZBTB41	275	10	14	285	95	96
79253161	stop gained	high	KIF14	275	10	14	285	95	96
79910536	synonymous	low	KLHL12	263	10	26	273	91	96
79120856	missense	moderate	ZNF281	236	9	54	245	82	96
80127163	synonymous	low	SYT2	232	9	58	241	81	96
76054628	synonymous	low	ASPM	282	11	6	293	98	96
76065369	missense	moderate	ASPM	282	11	6	293	98	96
79538778	missense	moderate	ENSBTAG00000053707	255	10	34	265	89	96
76110175	missense/splice region	moderate	ZBTB41	280	11	8	291	97	96
76519179	splice acceptor & intron	high	DENND1B	280	11	8	291	97	96
77649399	synonymous	low	PTPRC	280	11	8	291	97	96
75995210	missense	moderate	CFHR5	279	11	9	290	97	96
77649459	splice region/synonymous	low	PTPRC	279	11	9	290	97	96
78912172	synonymous	low	NR5A2	279	11	9	290	97	96
79563872	synonymous	low	KIF21B	253	10	36	263	88	96
77228184	splice region & intron	low	ENSBTAG00000049638	278	11	10	289	97	96

Exon analysis (SIFT software) indicates a deleterious effect for transcripts resulting from mutation in rs3423414874

More recent mutation in the HMW haplotype?



*Genotype status:
0 = noncarrier,
1 = carrier
2 = homozygous defect
3 = suspect carrier
4 = suspect homozygous



- **Tracing the affected calf's ancestor using pedigree data to a common bull born in 2008 (ROYLANE SOCRA ROBUST-ET).**
- **The available genomic data of the HMW haplotype were then sorted for the affected animals to separate carriers of the mutated MW haplotype for animals that carry the (unmutated) original MW haplotype**

Pedigree trace (mutant vs not-mutant) identifies the real carriers of the HMW recessive gene.

- Recumbency haplotype frequency by year.

Year	Haplotype code					
	Code 0 (%)	Code 1 (%)	Code 2 (%)	Code 3 (%)	Code 4 (%)	Total (%)
2000-2009	82726 (1.55)	1 (0)	0 (0)	0 (0)	0 (0)	82727 (1.55)
2010-2019	3079722 (57.85)	68043 (1.28)	327 (0.01)	203326 (3.82)	12398 (0.23)	3363816 (63.19)
2020-present	1577172 (29.63)	43255 (0.81)	324 (0.01)	230348 (4.33)	13382 (0.25)	1864481 (35.03)
Total (%)	4751877 (89.27)	111299 (2.09)	651 (0.01)	433674 (8.15)	25780 (0.48)	5323281 (100)

Both sides tracing to Robust

Both sides tracing to Robust,
but other sires have original version of HMW

*Genotype status:
0 = noncarrier,
1 = carrier
2 = homozygous defect
3 = suspect carrier
4 = suspect homozygous

Results: Haplotype vs gene test analysis

- Recumbency disorder gene tests[1] confirming most of the haplotype test results.

		Gene test results			
		# not carrier (%)	# carrier (%)	# recumbent (%)	Total (%)
Haplotype results	Code 0	675 (81.52)	3 (0.36)	0 (0)	678 (81.88)
	Code 1	0 (0)	56 (6.76)	0 (0)	56 (6.76)
	Code 2	0 (0)	0 (0)	2 (0.24)	2 (0.24)
	Code 3	67 (8.09)	14 (1.69)	0 (0)	81 (9.78)
	Code 4	3 (0.36)	8 (0.97)	0 (0)	11 (1.33)
	Total (%)	745 (89.98)	81 (9.78)	2 (0.24)	828 (100)

*Genotype status:
0 = noncarrier,
1 = carrier
2 = homozygous defect
3 = suspect carrier
4 = suspect homozygous

[1] Lab tests were shared by SelectSires to support this research

Results: Calf livability matched haplotype carrier status with old and new livability edits.

- Calf livability by Recumbency haplotype carrier status with old and new livability

Code*	Number	Livability (%)	Death age (mo)	Life (max=18 mo)
0	548,507	97.6	7.1	17.4
1	16,074	96.7	6.2	17.2
2	43	44.2	1.5	8
3	35,162	96.8	6.8	17.3
4	2,145	95.7	6	17.1
5	5,030,707	95.6	5.7	17.2

*Genotype status:
0 = noncarrier
1 = carrier
2 = homozygous defect
3 = suspect carrier
4 = suspect homozygous
5 = not genotyped but has livability record.

Conclusion:

- Revised haplotype tracking methods were applied in this study that achieved greater precision in identifying undesired recessive inheritance:
  - Utilizing sequence data to narrow the search to a single mutation candidate.
  - Sorting haplotypes into the original version vs. those containing a new mutation.
  - Requiring pedigree relatedness to the key ancestor affected animal.

- The new methods were initially tested on the haplotype for cholesterol deficiency (HCD) and were even more essential for recumbency, where the original haplotype is much more common than the haplotype containing the new mutation.

- Identification of harmful mutations and selection against them can improve fertility and reduce calf losses.

- Future developments:
  - *Haplotype* MW are yet not available to producers (expected before end of year)
  - Recent collaboration with HAUSA -> ~ 7,000 lab tests for preliminary analyses
  - Definition of the trait, naming and official recognition is pending.

Thank you for your attention

- Participating dairy producers supplied pedigree and genomic data
- Industry collaboration in data processing, handling and transfer: (N)DHIA, DPRC , PDCA, NAAB , Nominators, Genomic Labs
- Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by CDCB
- CDCB is an equal opportunity provider and employer

Questions?