

Integrating genomics, testing, and management strategies to control OPP

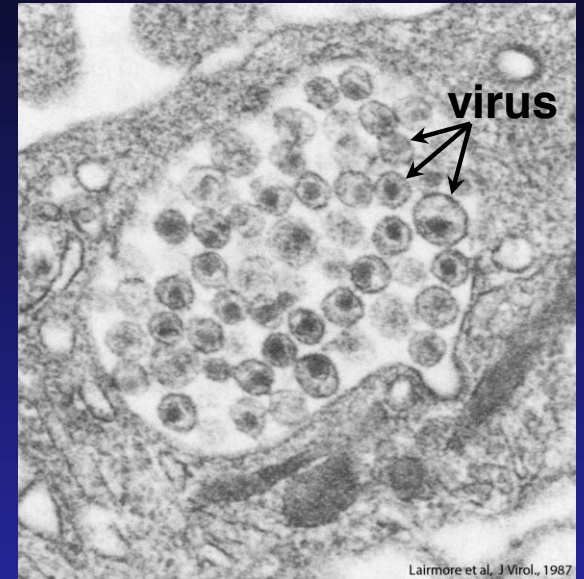


Kreg Leymaster
USDA, Agricultural Research Service,
U.S. Meat Animal Research Center,
Clay Center, NE

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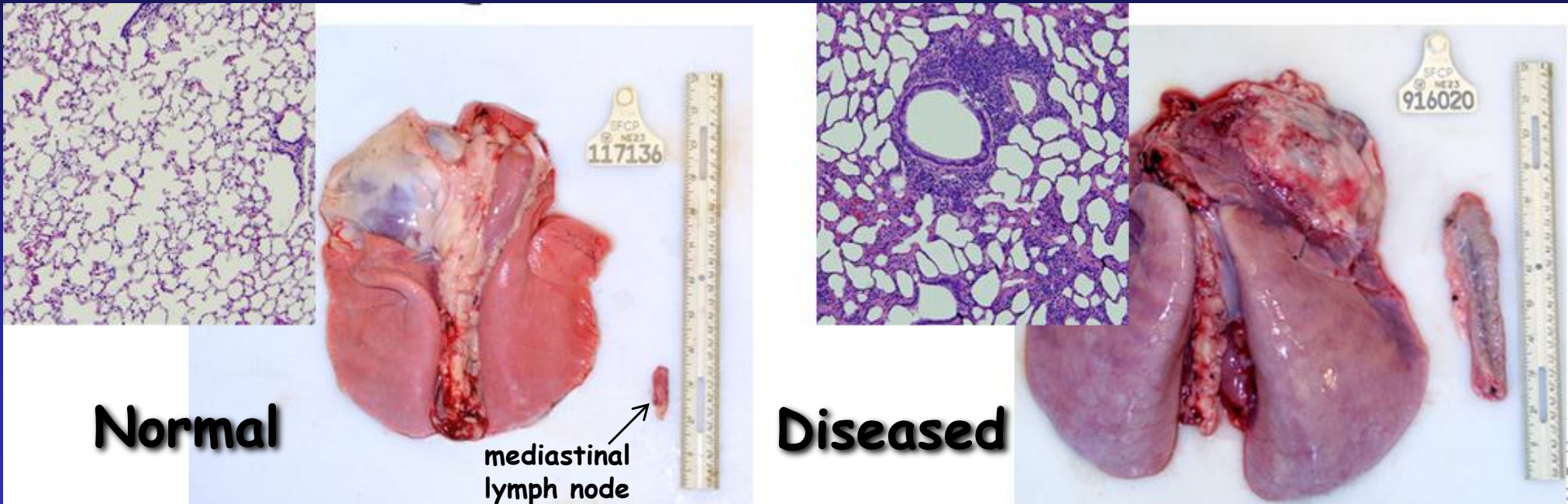
Ovine lentivirus

- **Visna/Maedi Virus (VMV) isolation in 1958**
 - Sigurdsson et al. J. Infect. Dis. 1964
- **Ovine Progressive Pneumonia Virus (OPPV)**
 - Kennedy et al, Virology 1968
- **The prototype "slow virus"**
- **Retrovirus, integrates into host genome**
 - sense strand RNA virus
- **Target cells are monocytes and macrophages**
 - When infected monocytes migrate into the interstitial spaces of affected organs, they mature into macrophages (white blood cells). This maturation is the trigger for transcription of integrated proviral DNA.
- **Affects lungs, central nervous system, lymph nodes, joints, and mammary glands.**



Alveolar macrophage infected with OPPV

Clinical OPP in adult sheep at USMARC



Normal

mediastinal
lymph node

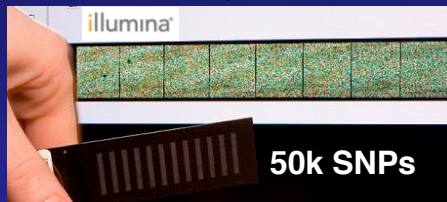
Diseased

Loss of weight
Labored breathing
Hard bag (irreversible)
Arthritis/lameness
Encephalitis (paralysis)

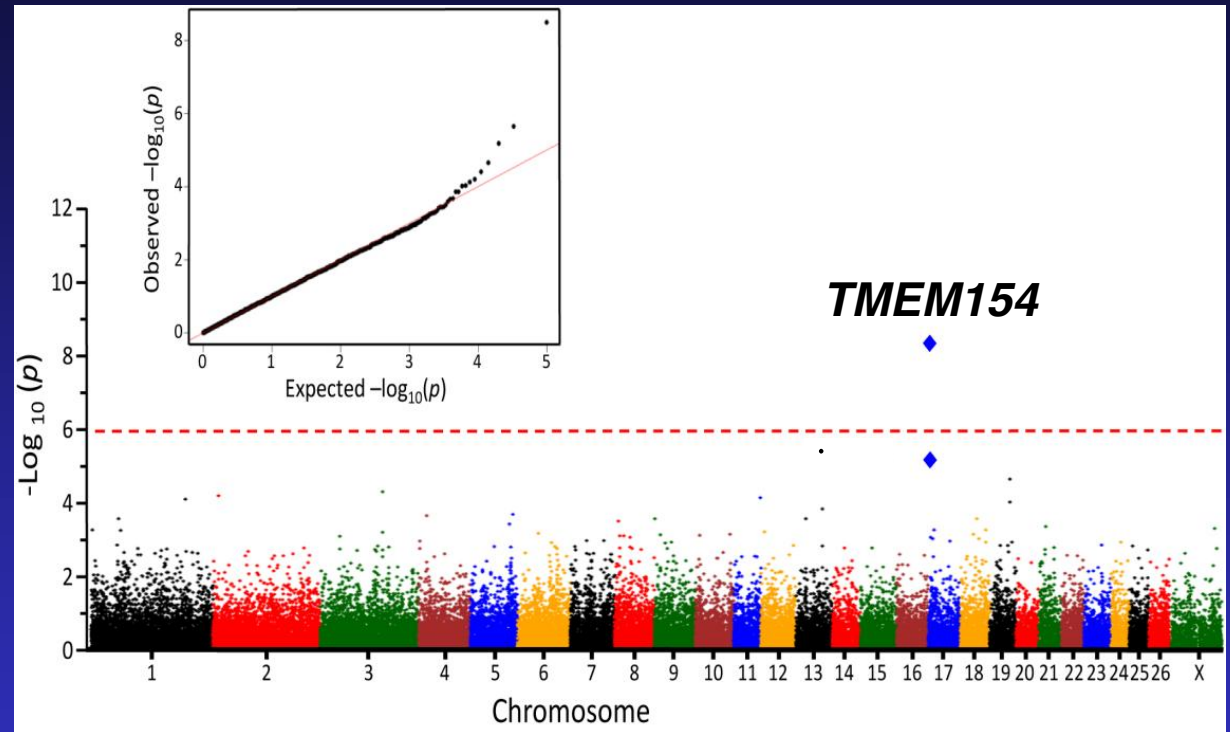
Cost of OPPV infection

- While few producers believe they have OPP, 36% of operations are infected (24% prevalence).
 - APHIS Veterinary Services, Centers for Epidemiology and Animal Health December, 2003
- Sheep are infected for life with no treatment or vaccines.
- Infected sheep often do not show clinical symptoms.
- OPPV-infected ewes:
 - are less likely to lamb,
 - wean 8% fewer lambs,
 - produce 20% less litter weaning wt per ewe exposed on an annual basis (Keen et al. 1997, Prev Vet Med, 155-169)
- Infected flocks require more replacement ewes.

Path to discovery of a gene affecting susceptibility to OPPV infection.



***TMEM154* gene**



OPEN ACCESS Freely available online

PLoS GENETICS

Reduced Lentivirus Susceptibility in Sheep with *TMEM154* Mutations

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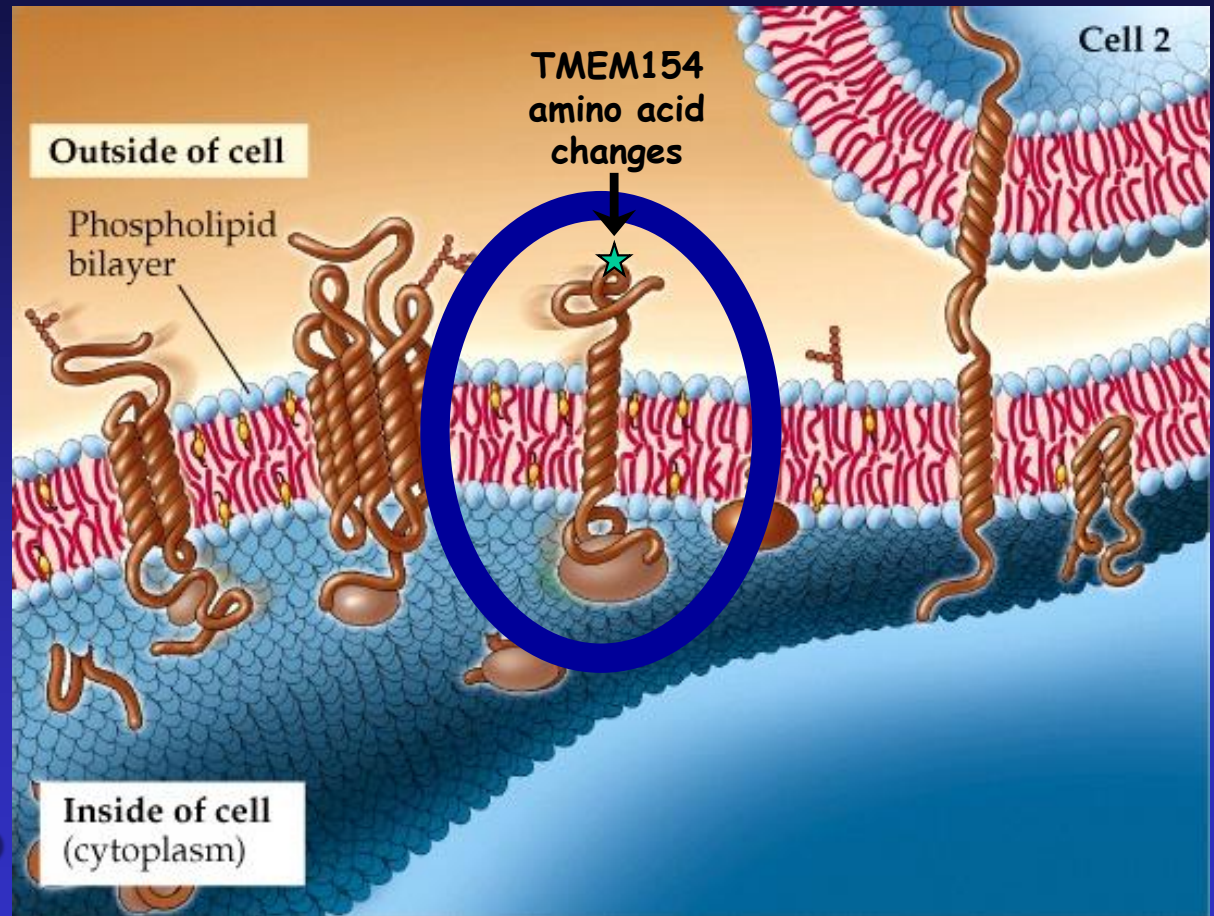
The *TMEM154* gene is predicted to encode a membrane protein

Membranes are the envelopes that surround animal cells.

Proteins do most of the work in cells and regulate the body's tissues and organs.

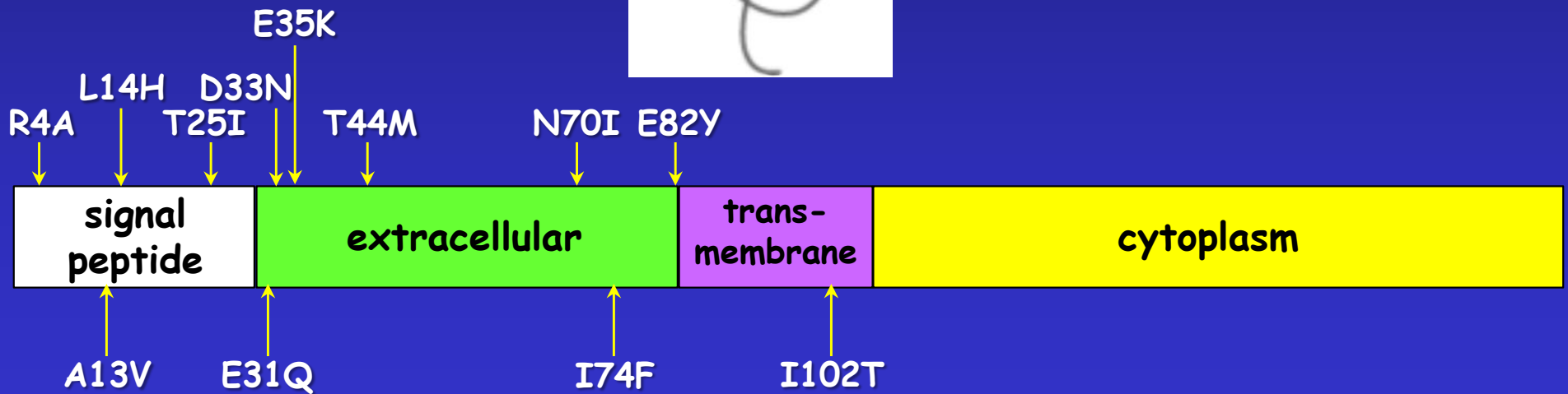
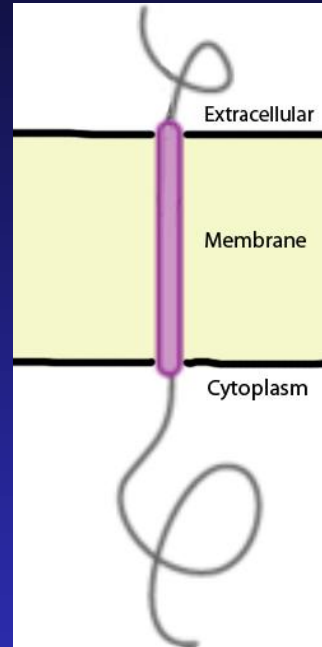
Amino acids are the building blocks of proteins.

Genes encode DNA sequences corresponding to amino acids in proteins.



Different versions of *TMEM154* are encoded in sheep and some versions are associated with greater susceptibility to infection.

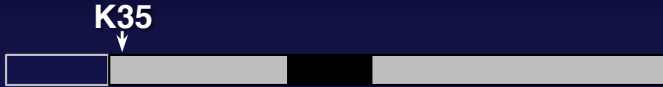
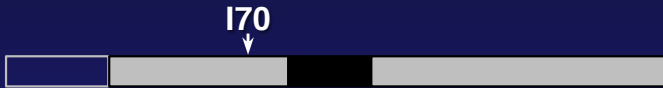



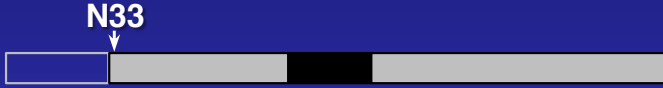


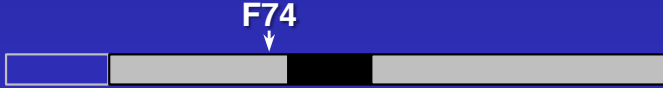
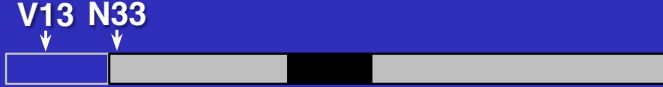

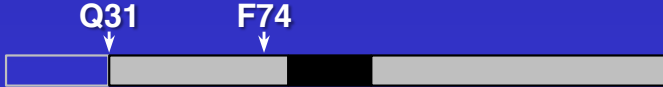
TMEM154 has amino acid changes at 12 codons.



A haplotype encodes a specific amino acid sequence.

Codon	Haplotypes		
	1	2	3
4	Arginine	-	-
13	Alanine	-	-
14	Leucine	-	-
25	Threonine	-	-
31	Glutamate	-	-
33	Aspartate	-	-
35	Lysine	Glutamate	Glutamate
44	Threonine	-	-
70	Asparagine	Isoleucine	-
74	Isoleucine	-	-
82	Glutamate	-	-
102	Isoleucine	-	-

Summary of various *TMEM154* haplotypes

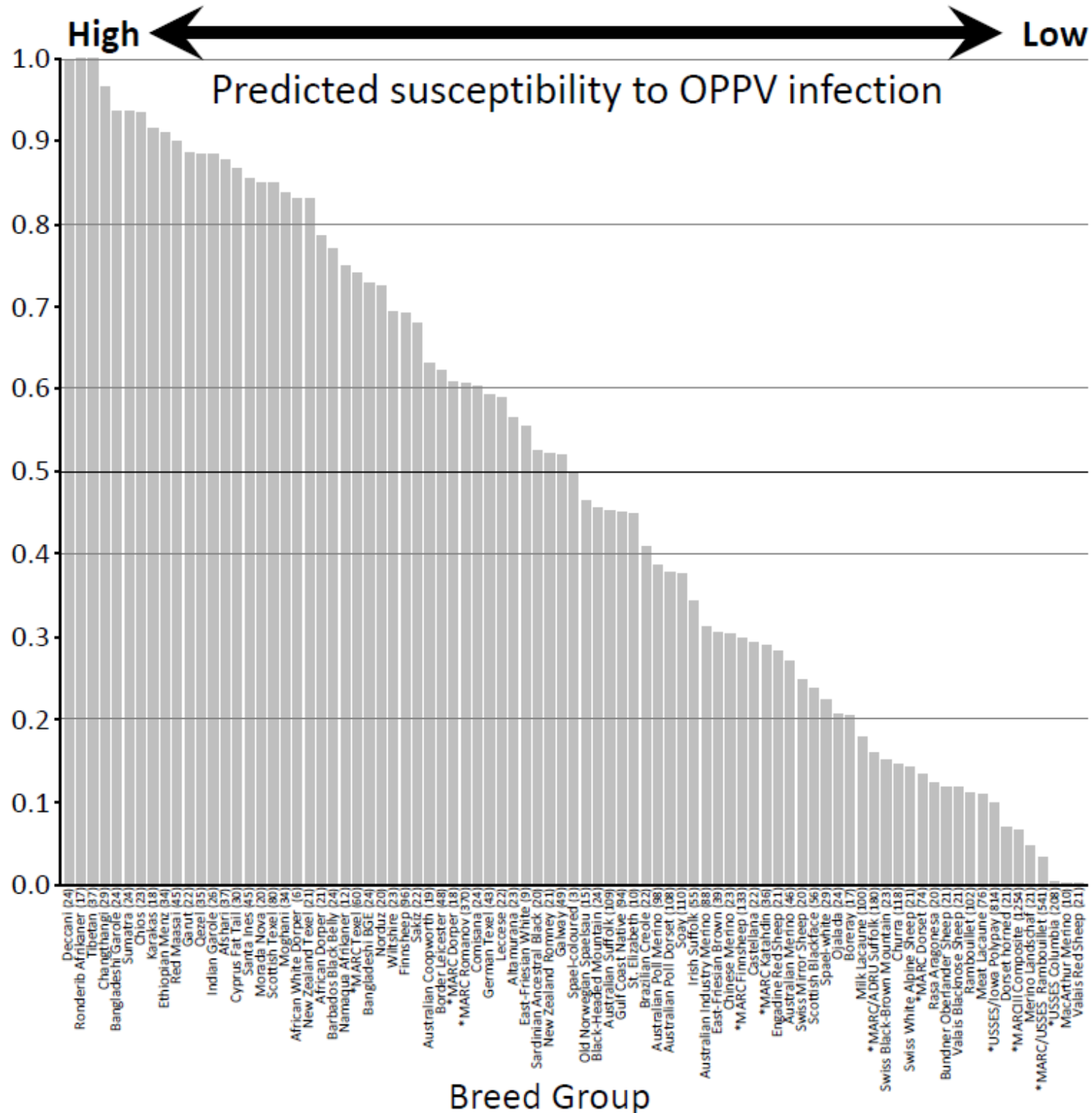
Haplotype	Protein structure	Effect	Frequency
1		Less susceptible	0.770
2		Highly susceptible	0.080
3		Highly susceptible	0.120
4		Unknown	0.020
6		Unknown	<0.001
9		Unknown	<0.001
10		Unknown	<0.001
11		Unknown	<0.001
12		Unknown	<0.001
13		Unknown	<0.001
14		Unknown	<0.001
15		Unknown	<0.001

Risk of infection is greater for sheep with haplotypes 2 or 3.

State	Age, yr.	No.	No. of breeds	Relative risk
NE	3	562	6	2.3
NE	4	225	8	4.9
NE	5	200	5	4.9
NE	6 - 9	376	6	5.1
NE	3 - 7	143	9	5.3
NE	4 - 5	280	1	3.8
ID	3 - 6	309	3	1.3
ID	3 - 5	392	3	1.8
IA	3 - 8	218	1	1.9

Overall, the infection rate of sheep with at least 1 copy of haplotype 2 or 3 was 2.8 times greater than sheep with 2 copies of haplotype 1.

Frequency of OAR17_5388531 "c" allele



Prospective experiments

Need well-designed studies that account for risk factors to advance understanding of transmission and to develop more effective methods of reducing the prevalence of OPP infection.

Experimental objectives

Test additive and dominance effects of haplotypes 1 and 3.
Study relative importance of maternal and non-maternal exposure.

Two primary routes of OPPV exposure

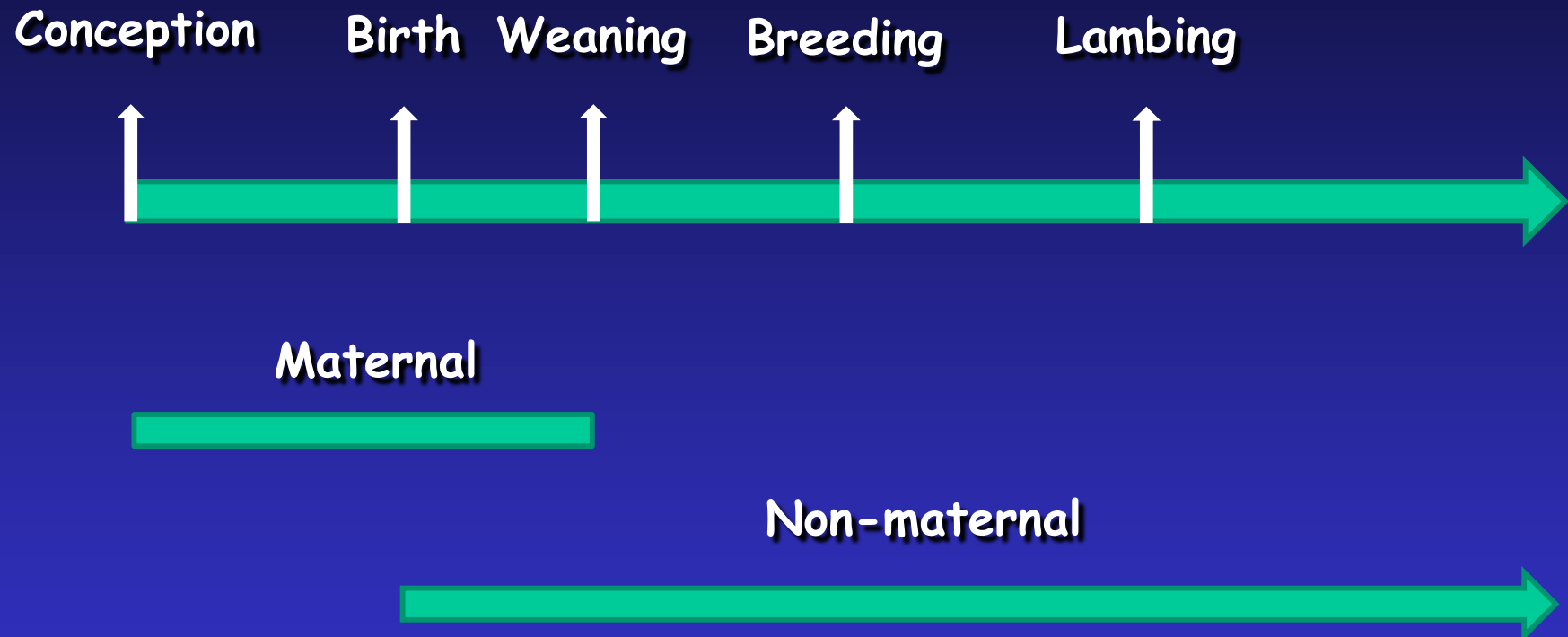
Maternal (vertical, dam-offspring)

Virus in colostrum and milk of dam

Non-maternal (horizontal, lateral)

Virus in lung secretions of flock mates

Biological model of OPPV exposure for breeding ewes



Experimental procedures

20 sentinel lambs were naturally reared by uninfected dams and 185 evaluation lambs were naturally reared by infected dams.

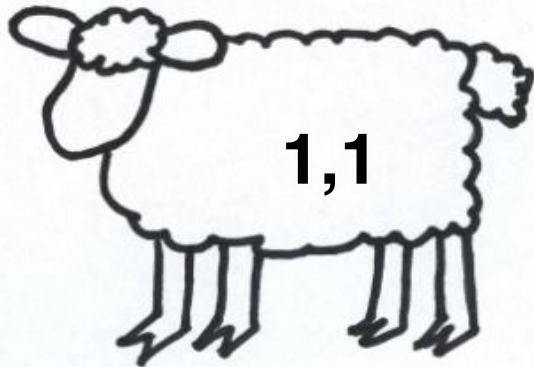
All dams and lambs were comingled.

All lambs were bled 1 week after weaning and every 5 weeks thereafter until about 9 months of age.

OPPV serological status was monitored by running cELISA assays in duplicate at USMARC.

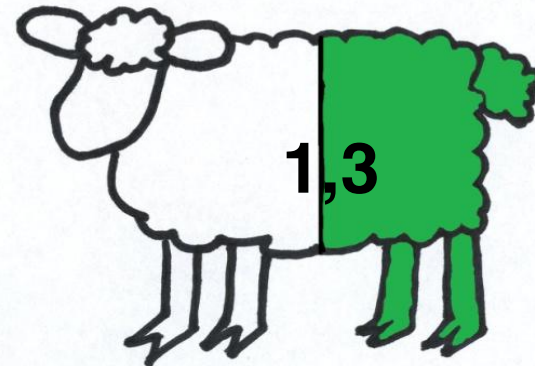
Sentinel lambs

Uninfected ewes

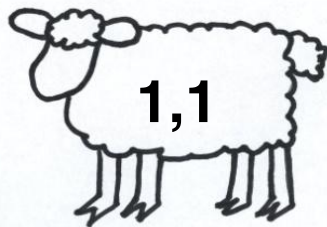


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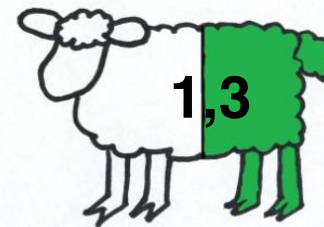
Rams



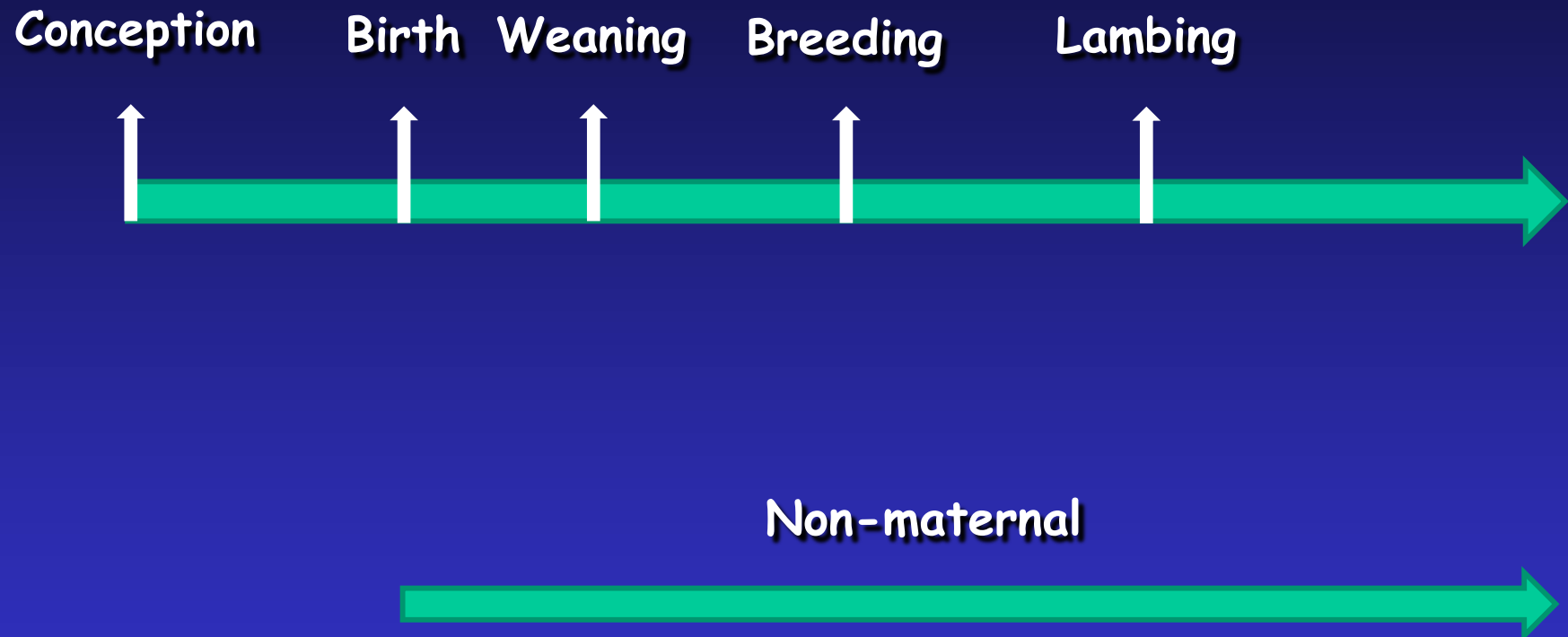
10 lambs



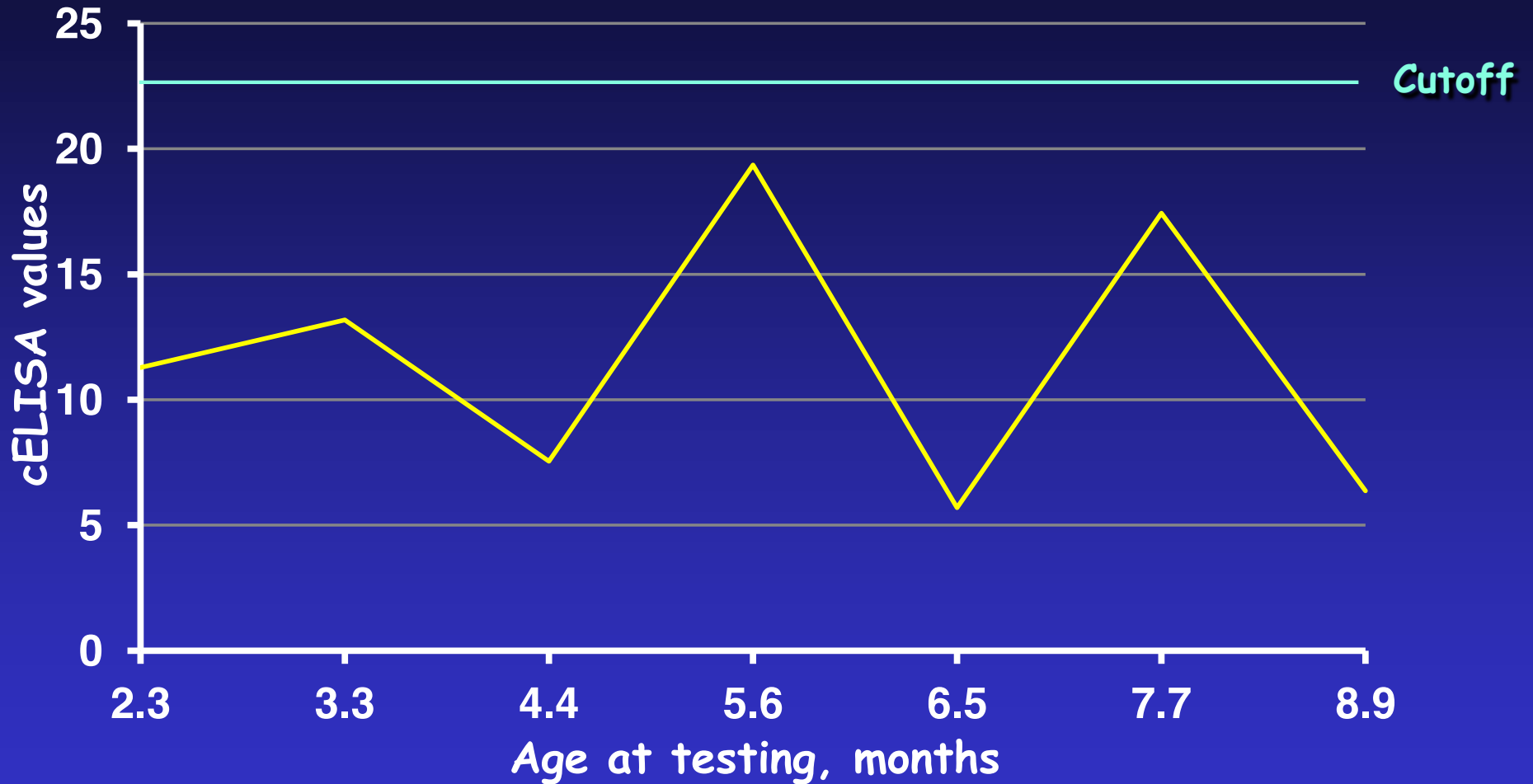
10 lambs



Biological model of OPPV exposure for sentinel lambs born to uninfected dams



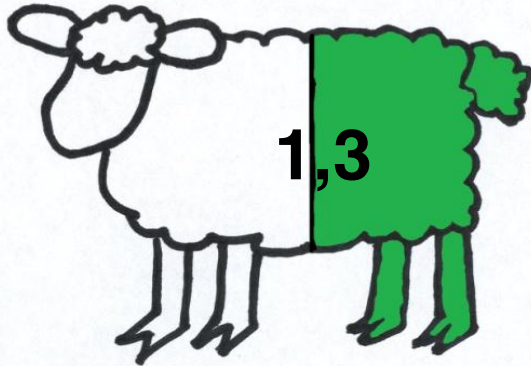
cELISA values for a typical sentinel lamb.



Non-maternal exposure caused little, if any, OPPV infection to 9 months of age.

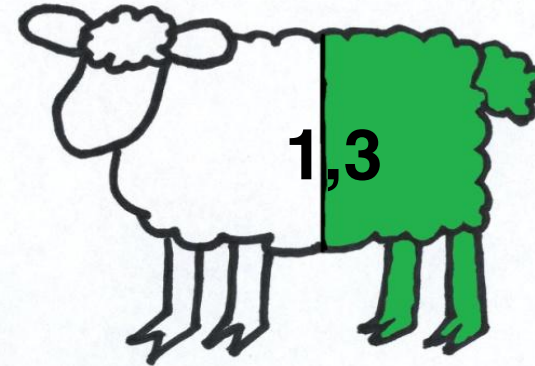
Evaluation lambs

140 infected ewes

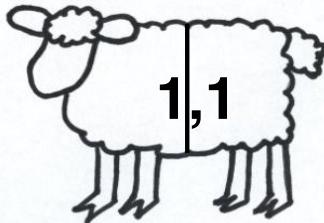


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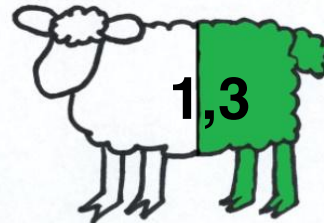
Rams



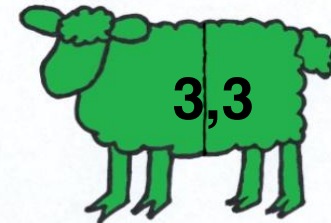
56 lambs



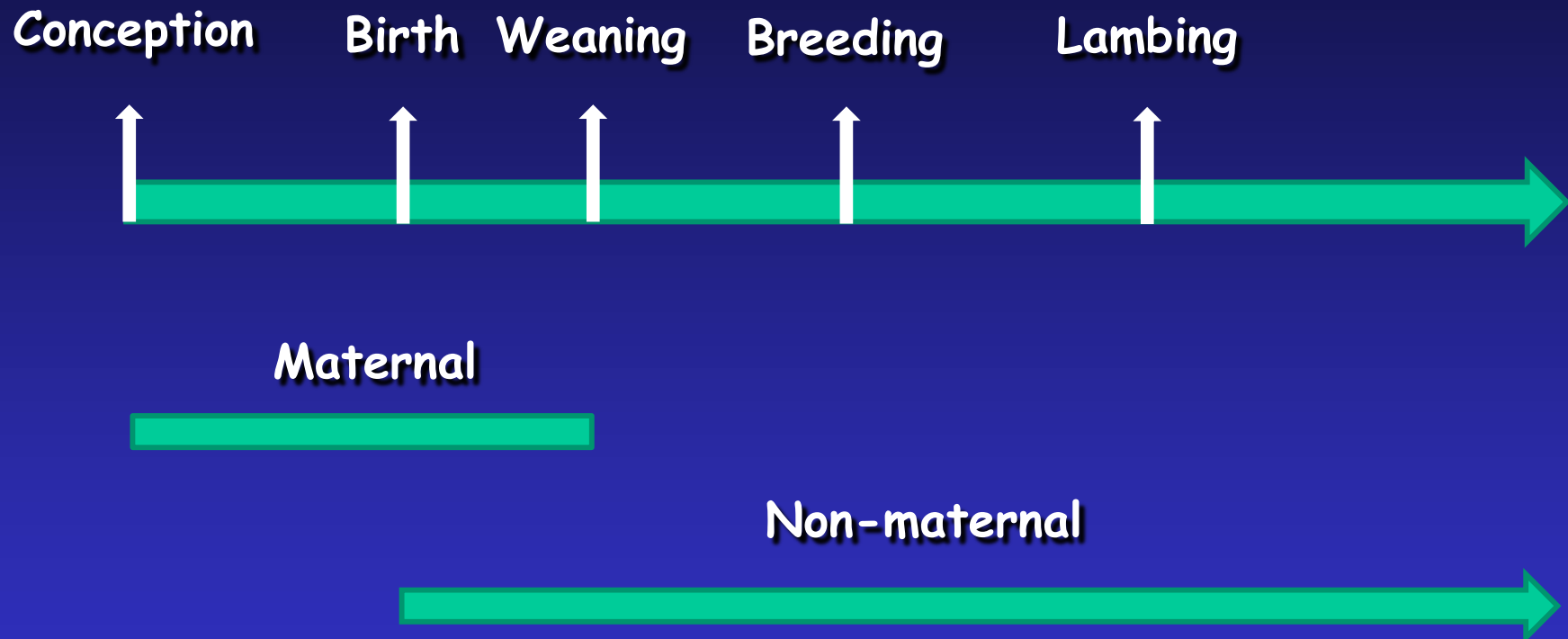
70 lambs



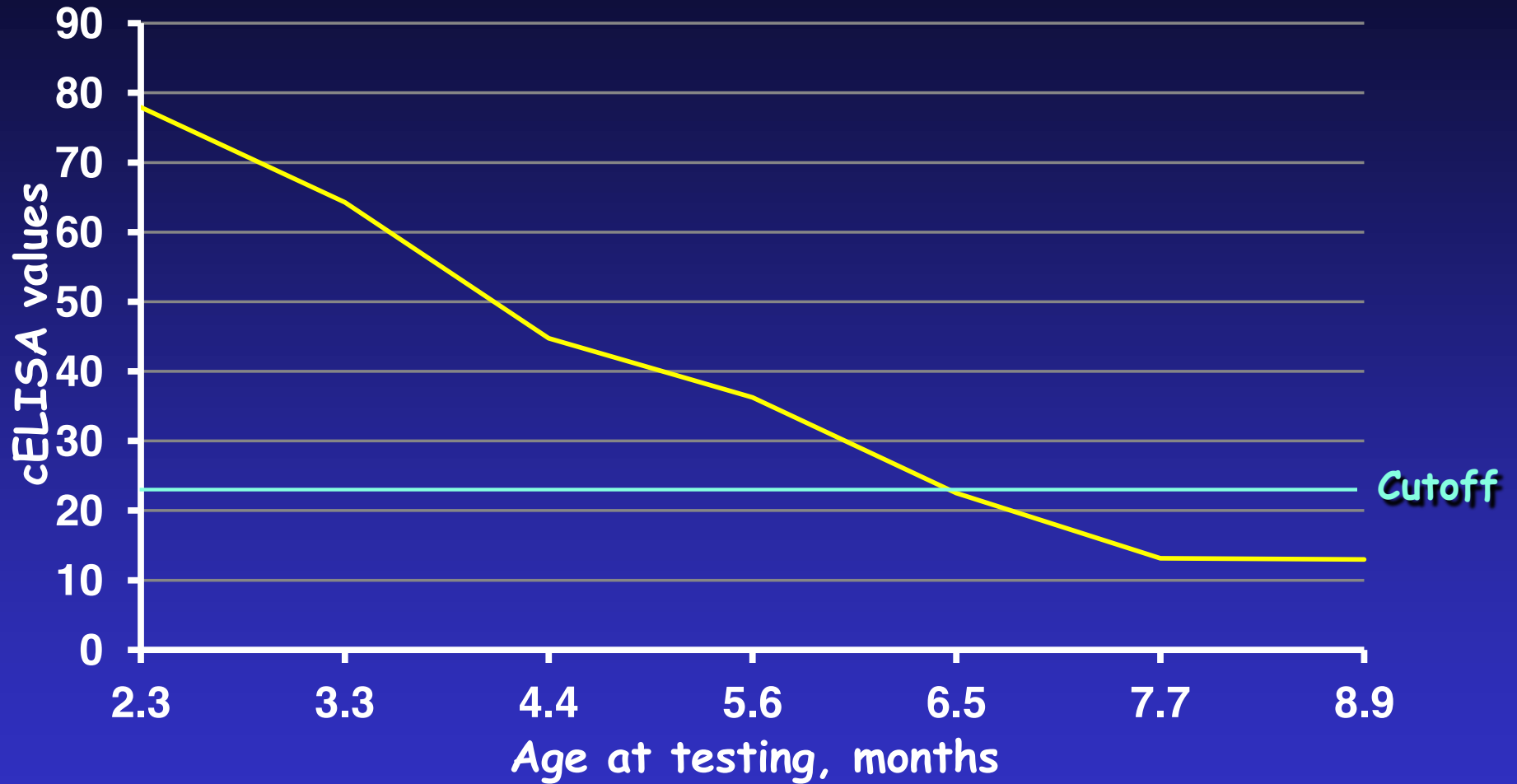
59 lambs



Biological model of OPPV exposure for evaluation lambs born to infected dams

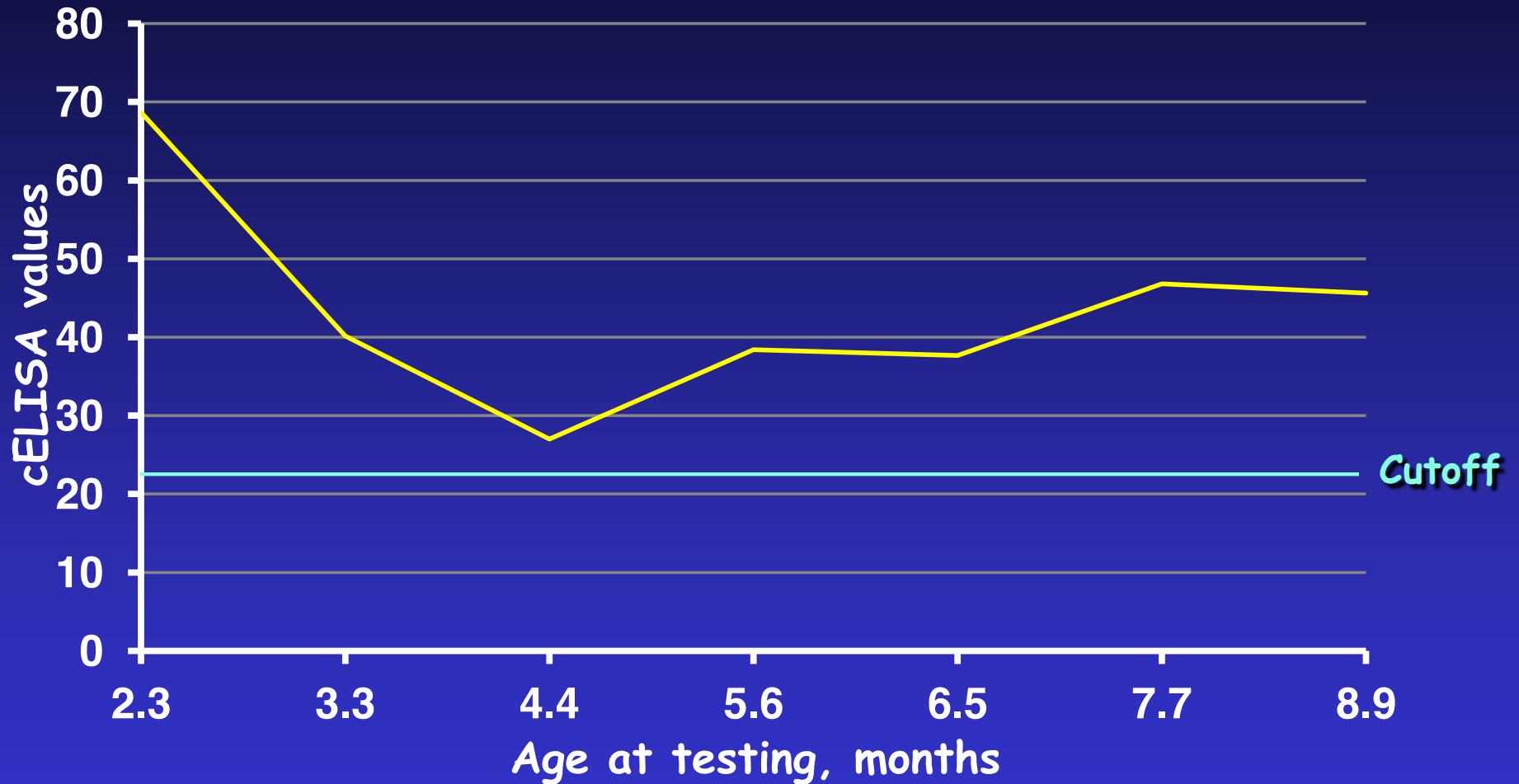


cELISA values for a typical seronegative lamb.

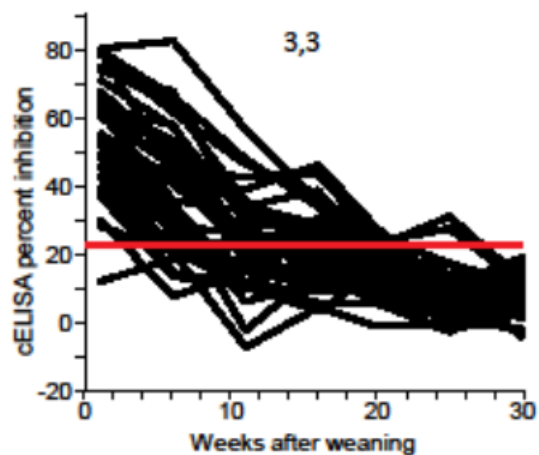
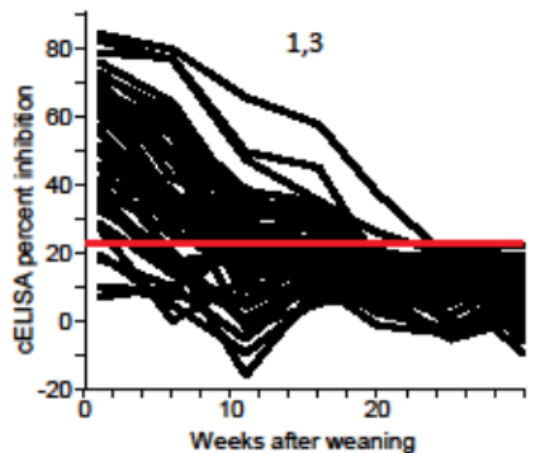
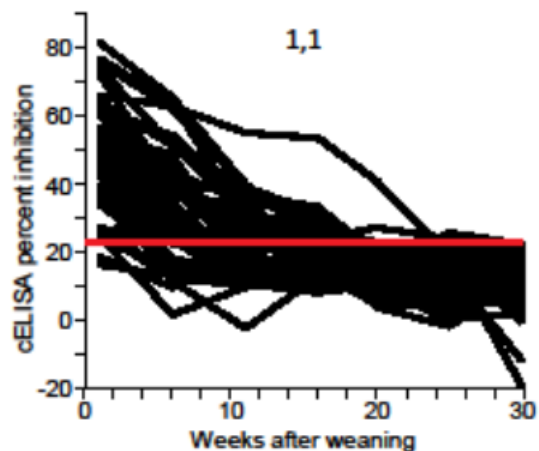


Trend shows the loss of maternal antibody,
with implications for age at testing.

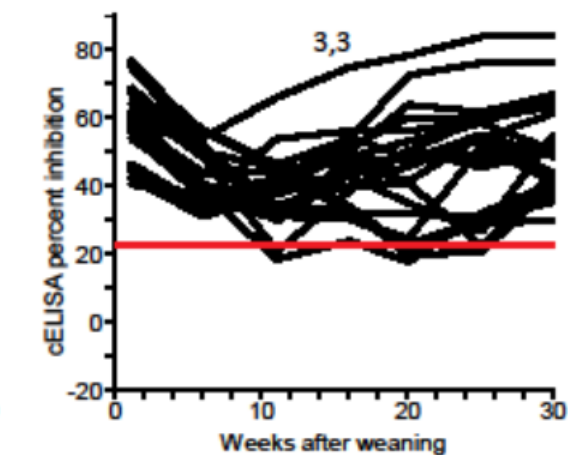
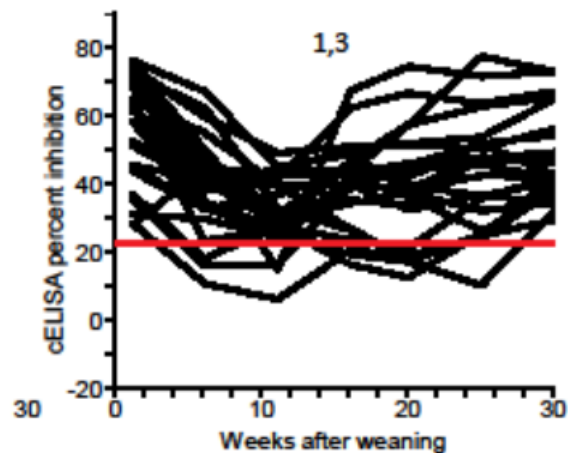
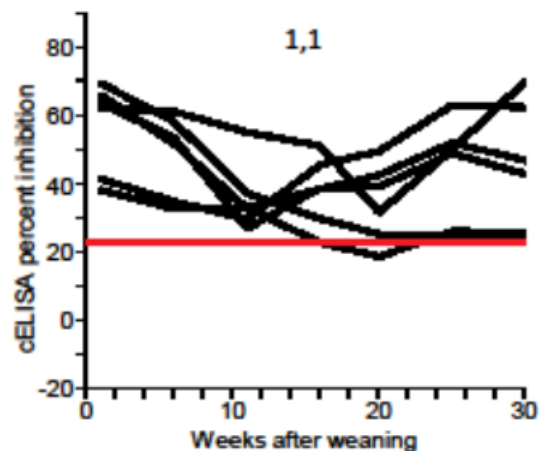
cELISA values for a typical seropositive lamb.



Uninfected



Infected



Frequency of diplotypes and OPPV serological status of naturally-exposed lambs at 9 months of age.

	Diplotype		
Serological status	1,1	1,3	3,3
Negative	50	47	38
Positive	6	23	21
Percent infected	10.7	32.9	35.6

All lambs were born to infected ewes, yet only 11% of 1,1's and 34% of 1,3's and 3,3's were infected.

The infection rate of lambs with 1 or 2 copies of haplotype 3 was 3.2 times greater than lambs with 2 copies of haplotype 1.

Haplotype 1 is recessive to haplotype 3.

Important results from this experiment.

Confirmed association of TMEM154 haplotypes with susceptibility.

Established that haplotype 1 is recessive to haplotype 3.

Non-maternal exposure caused little, if any, OPPV infection to 9 months of age.

Maternal exposure during the preweaning period infected, at most, 11% of genetically less-susceptible lambs and 34% of genetically more-susceptible lambs.

Therefore, the primary cause of infection in a flock of mature ewes must be due to non-maternal exposure that occurs after young ewes join the infected breeding flock.

The key management strategy is isolation of young ewes to prevent subsequent non-maternal exposure.

Conventional procedures to establish OPP-free flocks.

1. Periodically test all sheep and cull seropositive.
If testing annually, test 1 month before lambing.
Replace with offspring from seronegative ewes, preferably old ewes to exploit genetics for less susceptibility.
2. Artificially rear lambs and isolate from infected sheep.
3. Depopulate and repopulate with sheep from OPP-free flocks.

OPP-free flocks established through these approaches remain genetically susceptible to OPPV and will become infected if subsequently exposed to infected sheep.

Advice to manage impacts of OPPV infection

Use information to supplement, not replace, your current selection and culling procedures.

Determine serological status of flock, particularly older ewes.

Don't discard good genetics because of seropositive test results.

Don't automatically cull lambs born to infected ewes.

Know the TMEM154 diplotype of breeding rams.

Practical approach to reduce OPP prevalence in highly-infected flocks?

1. Put all ewes, infected and uninfected, into breeding - try to use rams with haplotype 1.
2. Bleed resulting ewe lambs at 7 months of age or older to determine serological status.
3. Keep seronegative ewe lambs isolated from infected flock.
4. Mate ewe lambs to rams that will increase the frequency of haplotype 1.

Note: We have not evaluated this approach at USMARC, but some producers are implementing it.

Submit by Email

Print Form

Date 1/11/13

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LINCOLN, NE 68521
402-435-0665
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Sheep Genetic Testing Submission Form

Farm/Name Vet Hospital/Name
 Address Address
 City City
 State Zip Code State Zip Code
 Email/Fax/Phone Email/Fax/Phone

Preferred Reporting Method: Email Fax Mail

Results are typically available 5 to 10 business days after sample arrival

TEST INFORMATION			
Test Code	Test Type	Cost Per Sample	
		1-999	1,000+
OPP	Ovine Progressive Pneumonia Virus Susceptibility Genetic Test	\$12	\$10
OPT	Ovine Parentage Testing	\$19	\$15
SCR	Ovine Scrapie Susceptibility Test	\$15	\$12
SIRE	Ovine Myostatin Genetic Testing	\$25	\$20
VIRT	Ovine Progressive Pneumonia Virus antibody detection (cELISA) from serum	\$6	\$5

Services provided are subject to GeneSeek's Terms and Conditions, which is available at <http://geneseek.com/terms.html>

PLEASE INCLUDE ANIMAL NSIP CODE IF POSSIBLE

SAMPLE INFORMATION							
Animal Name/ID	TEST CODE	TEST CODE 2	COST	Animal Name/ID	TEST CODE	TEST CODE 2	COST

Payment must be submitted with your samples. Please make checks payable to GeneSeek, Inc. **Total Amount Due:**

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Date Received:
 Received By:
 Order Number:

Check Number:
 Amount:

PAYMENT INFORMATION

Name/Signature Discover
 Credit Card Number Mastercard
 Expiration Date 3 Digit Security Code Visa

GeneSeek has run about 800 genetic tests for OPPV.

Sample ID	R4A	L14H	T25I	D33N	E35K	T44M	N70I	E82Y	Concat	Diplotype
007	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2003	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2004	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2006	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2011	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2018	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2020	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2024	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2025	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2033	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2035	I	T	C	G	R	C	A	I	ITCGRCAI	1,3
2039	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2044	I	T	C	G	R	C	W	I	ITCGRCWI	1,2
2045	I	T	C	G	R	C	W	I	ITCGRCWI	1,2
2046	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2048	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2053	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2054	I	T	C	G	R	C	A	I	ITCGRCAI	1,3
2056	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2058	I	T	C	G	A	C	A	I	ITCGACAI	1,1
2060	Q	T	C	G	R	Y	A	I	QTCGRYAI	1,4
621	I	T	C	G	A	C	A	I	ITCGACAI	1,1
YEL573	I	T	C	G	A	C	A	I	ITCGACAI	1,1
YEL700	I	T	C	G	A	C	A	I	ITCGACAI	1,1

Known issues

It's early in the research process and there is much to learn.

Adverse environmental conditions can cause high rates of OPPV infection regardless of *TMEM154* diplotypes.

- poor ventilation
- high humidity
- high density of sheep

Viruses have a high mutation rate and can adapt.

Some OPP strains seem to have evolved to more efficiently infect sheep with diplotype 1,1.

In flocks with "hot" strains of OPPV, selection for haplotype 1 may not significantly reduce the incidence of OPPV infection.

Acknowledgments

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