USMARC Update

Brad A. Freking
USDA, Agricultural Research Service,
U.S. Meat Animal Research Center,
Clay Center, NE

USDA is an equal opportunity provider and employer.

January 23, 2020

Status of Current Projects

- Romanov Genetic Selection / Augment Diversity
  2017-2022
- Evaluation of Maternal Lines under Pasture Lambing
  - 2014-2019
- Evaluation of Behavior under Barn Lambing
  - 2018-2020
- Katahdin Resource Population
  - 2020-
- Genomic Projects
  - Augment Rambouillet assembly
  - Romanov and White Dorper Trio-Binning haplotype resolved assemblies
  - Genotype by sequencing platform

- Update on 5-year OPP challenge studies

Genetic susceptibility to ovine progressive pneumonia (OPP)

Mike Heaton, Ph.D.
USDA Meat Animal Research Center (MARC), Clay Center, Nebraska
An equal opportunity provider and employer

Acknowledgments

USDA MARC scientists

Carol Chitko-McKown, PhD
Mike Clawson, PhD
Brad Freking, PhD
Greg Harhay, PhD
Shuna Jones, DVM
Tim Smith, PhD
Kreg Leymaster, PhD

Collaborating scientists

USDA, Animal Disease Research Unit, Pullman, WA
Lynn Herrmann-Hoesing, PhD, Don Knowles, PhD, Stephen White, PhD

USDA, Sheep Experiment Station, Dubois, ID
Greg Lewis, PhD, Michelle Mousel, PhD

University of Wyoming, Laramie, WY
Will Laegreid, DVM, PhD

University of Louisville, Louisville, KY
Ted Kalbfleisch, PhD

GeneSeek, Lincoln, NE
Dustin Petrik, PhD, Barry Simpson PhD

Embrapa Goats and Sheep
Lucia H. Sider, DVM, PhD

Livestock Industries, CSIRO, Brisbane, Australia
James Kijas, PhD

The International Sheep Genome Consortium

OPP Natural Challenge Experiments

Table 1. Information about the three sets of evaluation ewes.

Exp.	Pop. flock	Diplotypes	Breed Composition[a]	Birth year	Years of evaluation
1	OP/13	11, 13, 33	1/2 RV, 1/4 RB, 1/8 WD, 1/8 KT	2011	2012-2016
2	OP/23	12, 13, 22, 23, 33	1/2 RV, 1/4 RB, 1/8 WD, 1/8 KT	2012	2013-2017
				2013	2014-2018
				2014	2015-2019
3	OP/14	11, 14, 44	1/2 RB, 1/2 KT	2013	2014-2018
				2014	2015-2019
				2015	2016-2019

[a]RV = Romanov, RB = Rambouillet, WD = White Dorper, and KT = Katahdin.

Topics

- The major sheep gene (*TMEM154*) affecting OPP
- Impact of *TMEM154* discovery and genetic testing
- Update on 5-year OPP challenge studies with ewes

The diseases caused by ovine lentiviruses are prevalent around the world

1915 South Africa: Graaff-Reinet disease

Mitchell, D.T. , 1915 . Union of South Africa. Dept. of Agric. 3rd and 4th Reports of the Director of Veterinary Research., Nov pp. 583-614 pp.

1923 USA: Montana Sheep Disease

Marsh, H., 1923. J. Am. Vet. Med. Assoc., 62:458-73

1939 Iceland: Visna and Maedi diseases

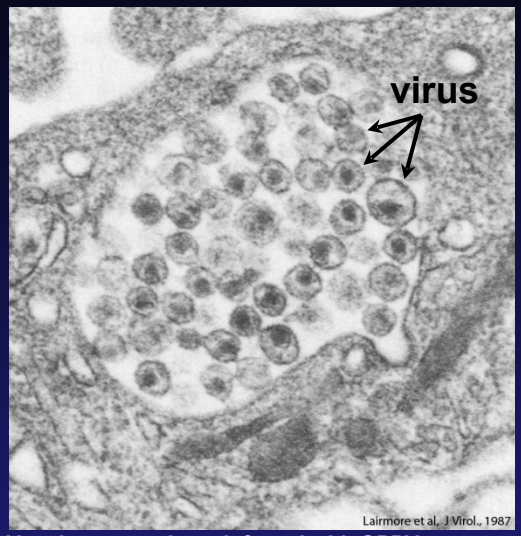
Gíslason, G., 1947. Iceland Dept. Ag.Publ. Reykjavik, pp. 235-57.

1942 France: La Bouhite

Lucam, F., 1942. Rec. méd. vét. 118: 273–284.

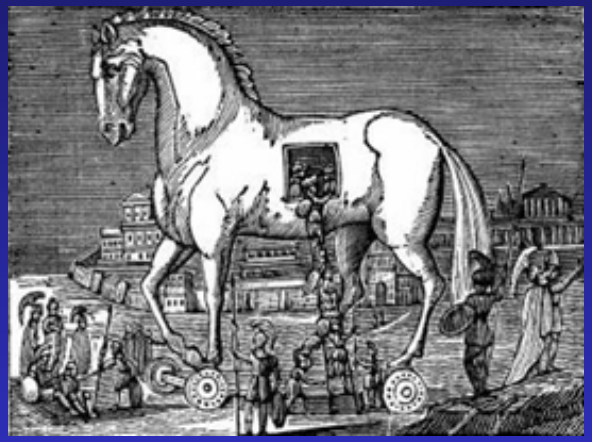
What is ovine lentivirus?

- The prototype “slow virus”
  - long incubation period
  - gradual onset of symptoms
  - irreversible and terminates in death



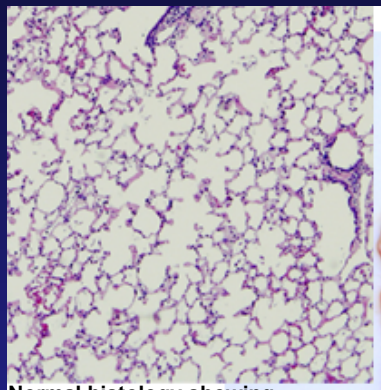
Alveolar macrophage infected with OPPV

- “Trojan horse” model of infection
  - The virus infects circulating white blood cells
  - The viral DNA inserts into the sheep DNA
  - When the infected white blood cell arrives at a site of inflammation, the virus “breaks out”.

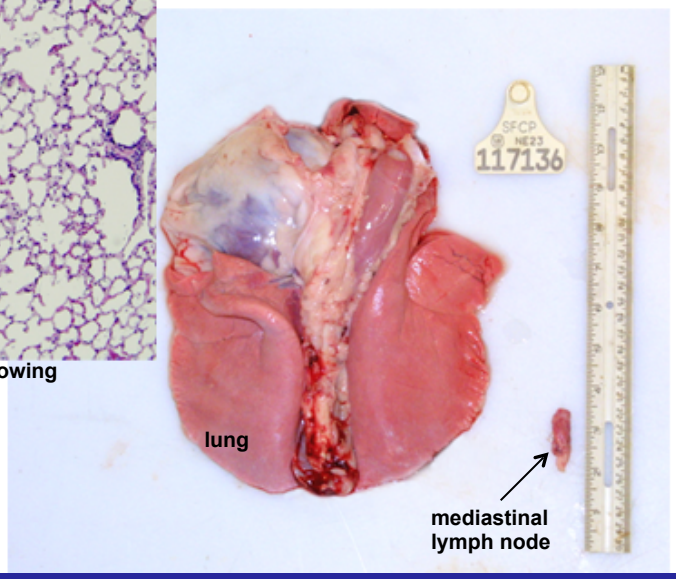

A 19th century engraving of the fabled Trojan Horse

Clinical OPP in adult sheep at USMARC

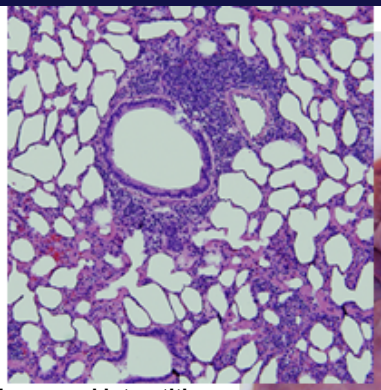
Normal



Normal histology showing alveolar sacs



Diseased



Increased interstitium

interstitial pneumonia

Transmission of the OPP virus

Primarily among ewes during lambing season by (~75%) via aerosolized respiratory secretions

Also from dam to offspring via infected colostrum (~25%)

Infections are life-long with no treatments or vaccines.

Cost of OPP in the USA

- 36% of sheep operations are infected
  - APHIS Veterinary Services, Centers for Epidemiology and Animal Health December, 2003

- Infected ewes:
  - are significantly less likely to lamb
  - wean 8% fewer lambs
  - Wean litters that are 24% lighter
  - $11 per ewe in milk replacer
    - Schwebach and Schwebach, Shepherd Magazine, July 2014

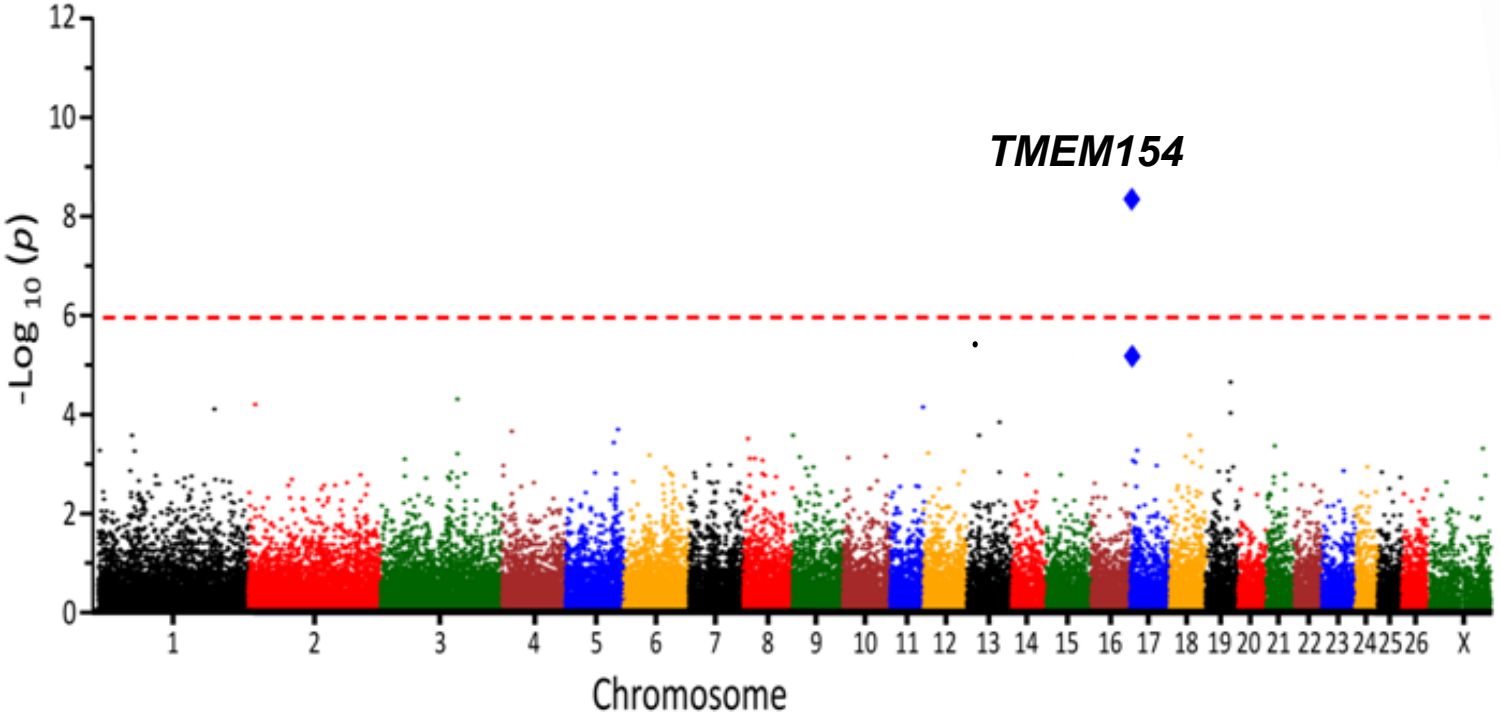
- Infected flocks require frequent replacement of ewes.
- Our goal has been to reduce, and then eliminate OPP.

In 2012, USMARC reported the discovery of *TMEM154* as a major gene affecting OPP

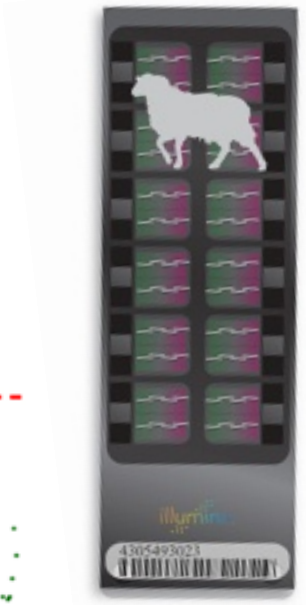
OPEN ACCESS Freely available online January 26, 2012 PLoS GENETICS

Reduced Lentivirus Susceptibility in Sheep with *TMEM154* Mutations

Michael P. Heaton[1]*, Michael L. Clawson[1], Carol G. Chitko-Mckown[1], Kreg A. Leymaster[1], Timothy P. L. Smith[1], Gregory P. Harhay[1], Stephen N. White[2], Lynn M. Herrmann-Hoesing[2], Michelle R. Mousel[3], Gregory S. Lewis[3], Theodore S. Kalbfleisch[4], James E. Keen[5], William W. Laegreid[6]

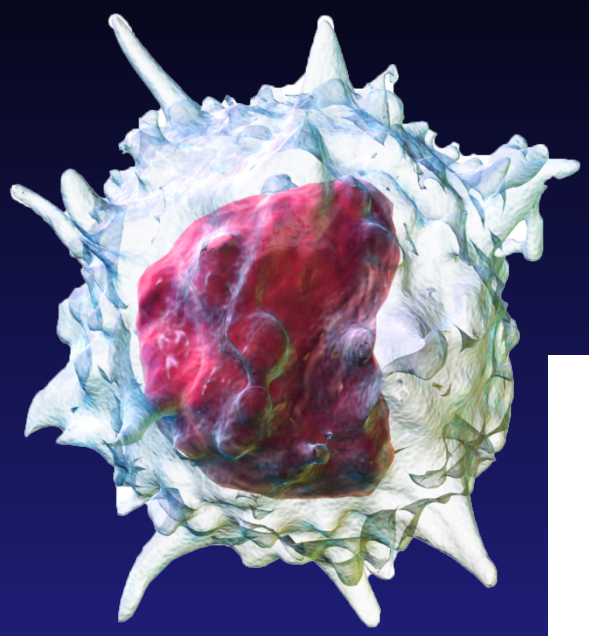


ISGC International Sheep Genomics Consortium

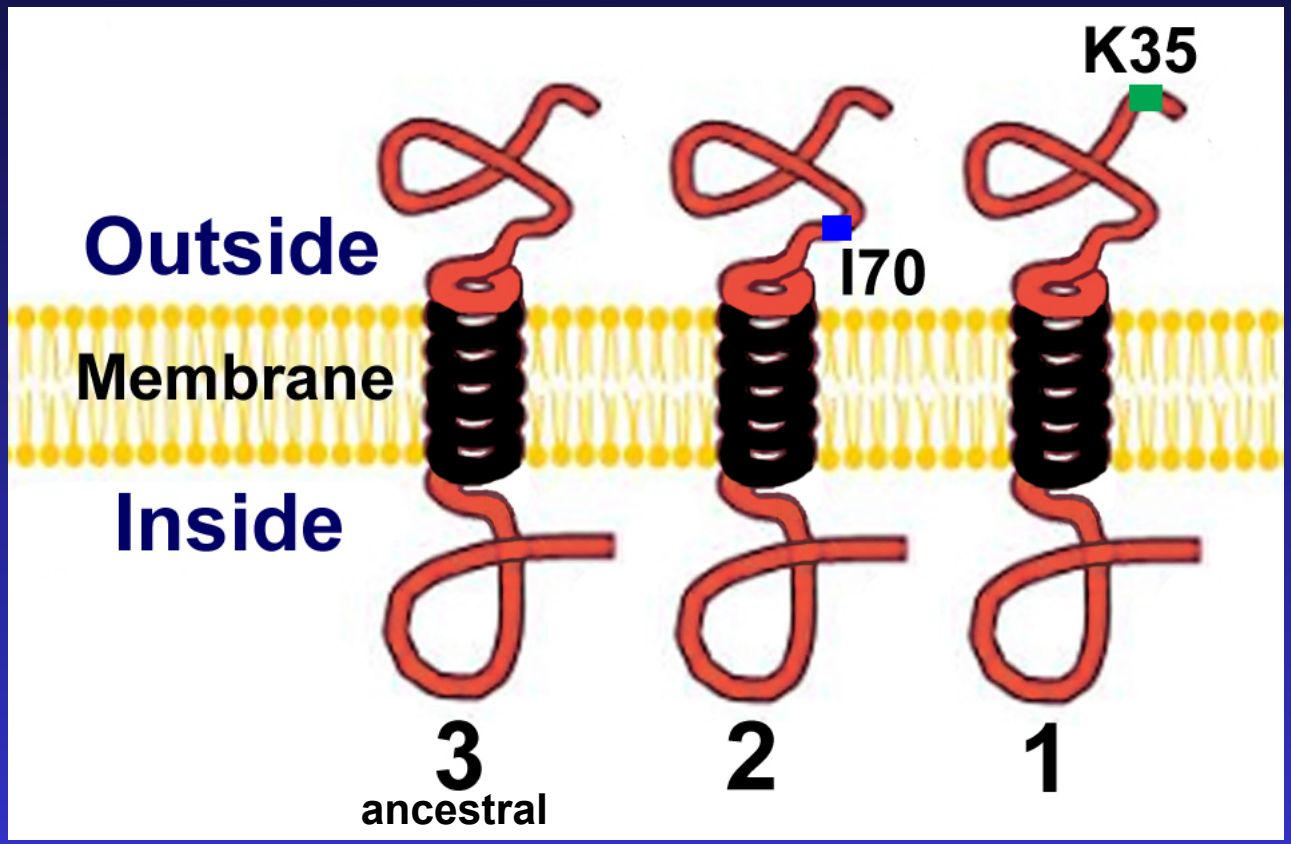


50,000 DNA markers

Sheep have three common variants of the TMEM154 protein



White blood cell (monocyte)



"Medical gallery of Blausen Medical 2014".

The doorway model for TMEM154 function

Biological cells have many “doors” for entry.

Viruses have to get inside the cell to replicate.

Evidence suggests that:

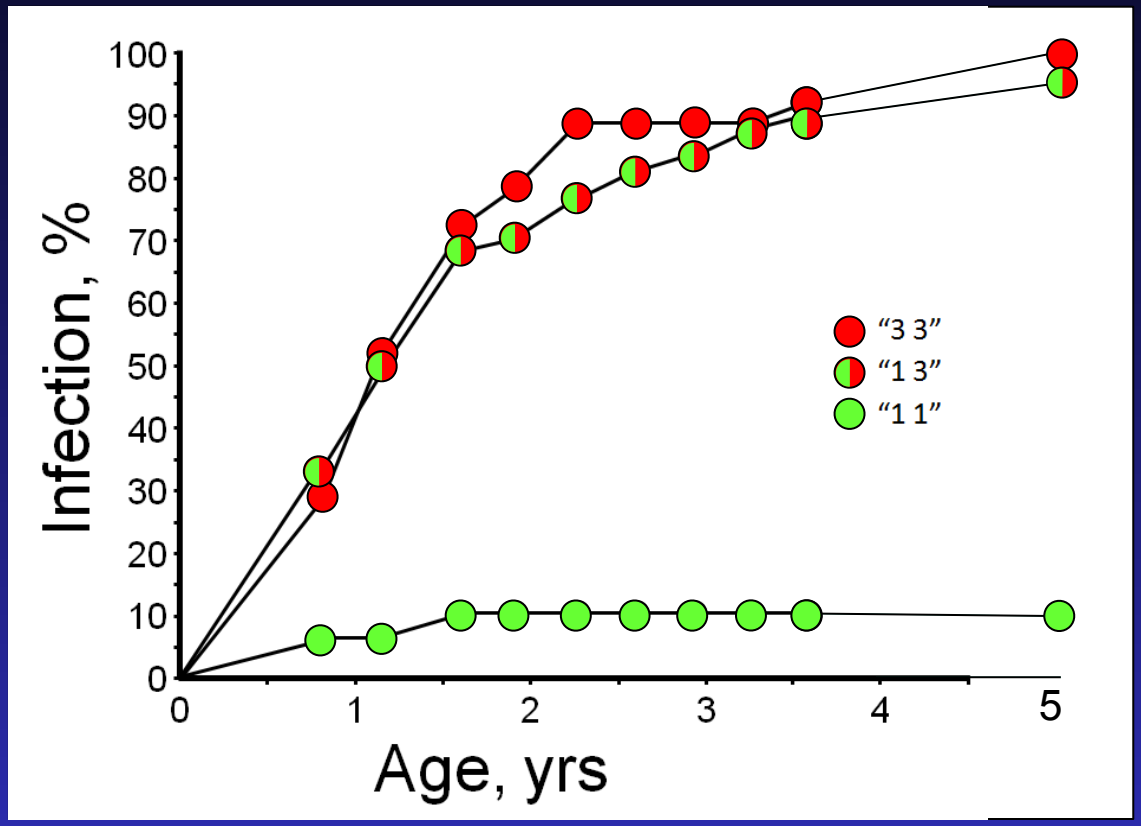
TMEM154 is like a handle on a cellular doorway for the virus to enter.

The K35 variant may reduce the virus’s grip on the door handle.

TMEM154 variants effect susceptibility to infection

Variant	Susceptibility	Feature
1	Low	K35
2	High	I70
3	High	Ancestral
4	Low	R4A(Δ53), M44
6	Low	I25, Y82(Δ82)
9	High	N33
10	Low	H14, K35
11	High	I25
12	High	F74
13	High	V13, N33
14	High	T102
15	High	Q31, F74

Effect of *TMEM154* variants in naturally-exposed ewes



97% infected at five years
(1 uninfected ewe)

Relative risk 11.1 (CI_{95} 3.8 to 21)

9% infected at five years

The infection rate was increased 11 times for an animal with a highly-susceptible genotype

Two additional just completed experiments

- Evaluate susceptibility of haplotypes 2 and 3.
- Evaluate susceptibility of haplotypes 1 and 4.

TMEM154 variants effect susceptibility to infection

Variant	Susceptibility	Feature
1	Low	K35
2	High	I70
3	High	Ancestral
4	Low	R4A(Δ53), M44
6	Low	I25, Y82(Δ82)
9	High	N33
10	Low	H14, K35
11	High	I25
12	High	F74
13	High	V13, N33
14	High	T102
15	High	Q31, F74

How can producers benefit?

First determine if your flock is infected

- Test some of your oldest ewes for infection
  - serological test, $6/animal)

Variant 1

Variants 4, 6, 10

Variants 3 and 2

Variant 9

OPEN ACCESS Freely available online PLOS ONE

Genetic Testing for *TMEM154* Mutations Associated with Lentivirus Susceptibility in Sheep

Michael P. Heaton[1*], Theodore S. Kalbfleisch[2,3*], Dustin T. Petrik[4], Barry Simpson[4], James W. Kijas[5], Michael L. Clawson[1], Carol G. Chitko-McKown[1], Gregory P. Harhay[1], Kreg A. Leymaster[1], the International Sheep Genomics Consortium[¶]

GENESEEK a Neogen Company

$10 to $12/animal

What about virus strains and environment?

- Research with German, Iranian, and Turkish flocks showing the similar *TMEM154* effects with visna maedi (VM) virus

Infection was 3-times greater for ewes with highly-susceptable *TMEM154* variants

CI_{95} 1.3 to 8.7, *p*-value 0.009

Endemic disease with less progression

- Rambouillet and Columbia breeds are nearly all “1,1” yet highly infected.
- Additional host genes may confer susceptibility
- Extensive management practices can reduce transmission

Progression of Disease in less susceptible genotype 4/4 ewes ?

- 8 ewes homozygous for 4 allele present at end of experiment were euthanized to examine lung phenotypes
- Four were serologically positive and four were negative

Progression of Disease in less susceptible genotype 4/4 ewes ?

Progression of Disease in less susceptible genotype 4/4 ewes ?

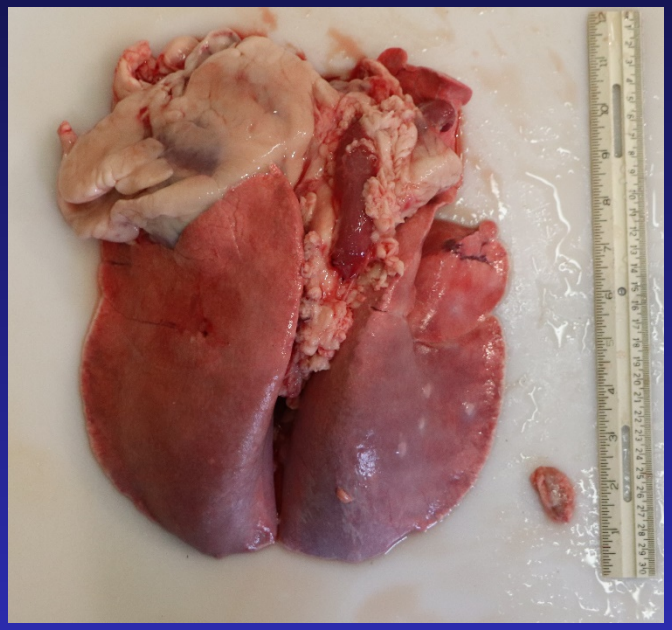
ID = 1473123
Serologically Negative

ID = 1573135
Serologically Positive

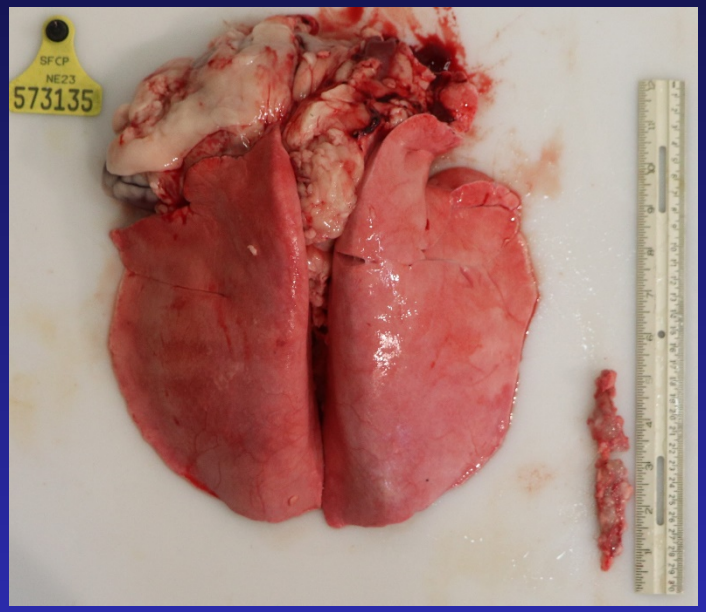
Positive at 17 months old
Euthanized 40 months after conversion

Progression of Disease in less susceptible genotype 4/4 ewes ?

ID = 1473123
Serologically Negative

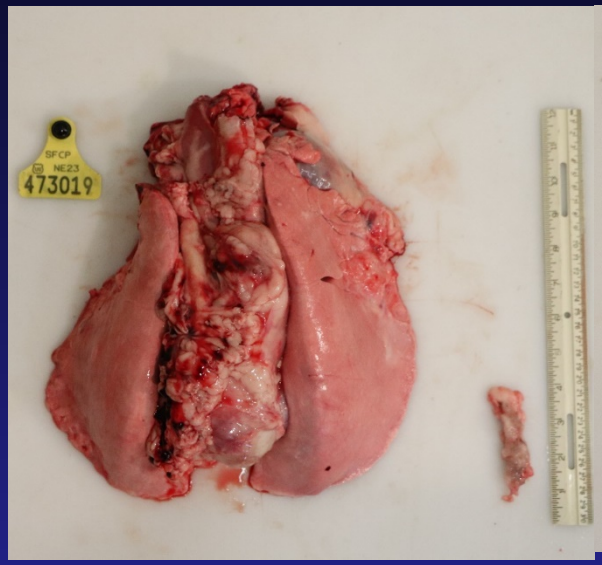


ID = 1573135
Serologically Positive

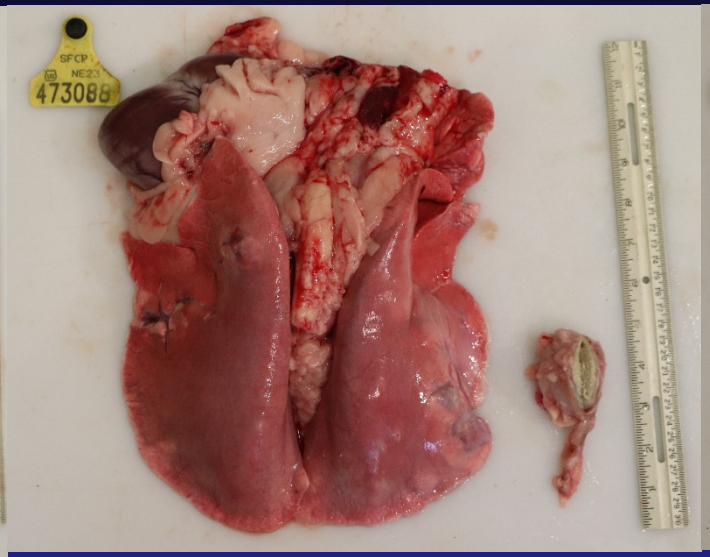


Positive at 17 months old
Euthanized 40 months after conversion

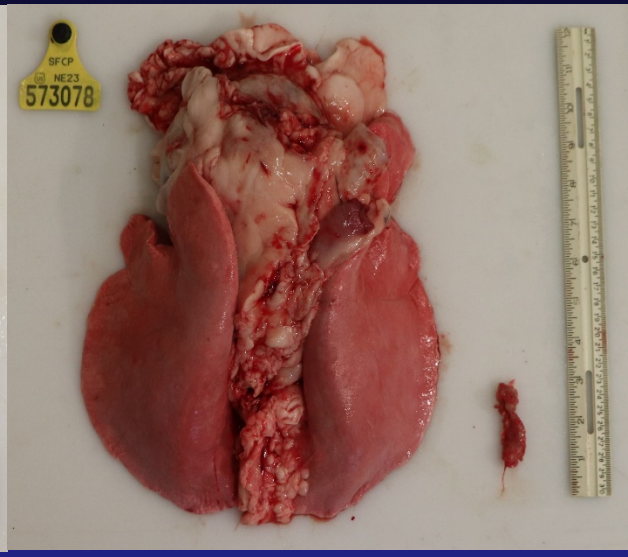
Progression of Disease in less susceptible genotype 4/4 ewes ?



ID = 1473019
Positive for 33 Months



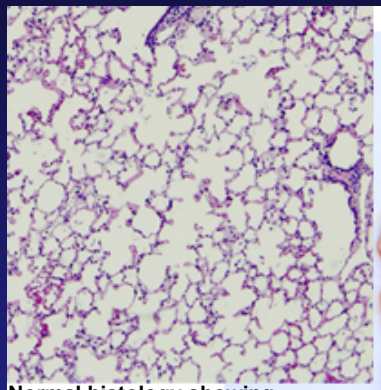
ID = 1473088
Positive for 21 Months



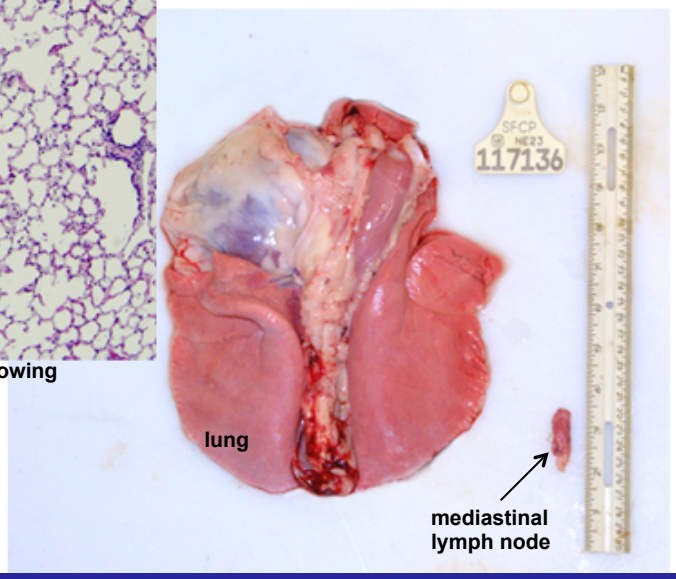
ID = 1573078
Positive for 21 Months

Clinical OPP in adult sheep at USMARC

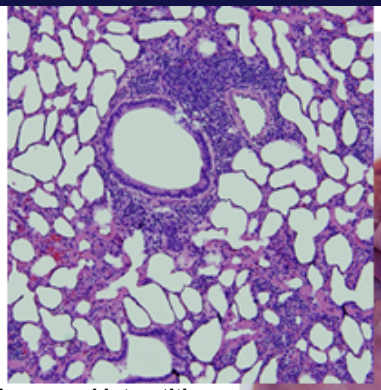
Normal



Normal histology showing alveolar sacs



Diseased



Increased interstitium

interstitial pneumonia

Our current general recommendation to reduce or eradicate OPP in a flock is the following:

1. Serologically test a random sample of the oldest ewes to determine prevalence of OPP.

2. Keep all productive ewes, regardless of infection status, for breeding.

3. Mate to rams with 1 or 2 copies of haplotype 1.
4. Naturally rear the resulting lambs and serologically test replacement ewe lambs at 7 mo of age or older.
5. Permanently isolate seronegative ewe lambs from the infected flock.
6. Mate ewes in the seronegative flock to rams that will increase the frequency of haplotype 1.
7. Monitor infection status in the seronegative flock by serologically testing the oldest ewes.
8. Progression of the disease appears to be slower in less susceptible genotypes

Leymaster et al. 2015