Mycoplasma ovipneumoniae: Highlights of Research and Investigative Findings in Alaska

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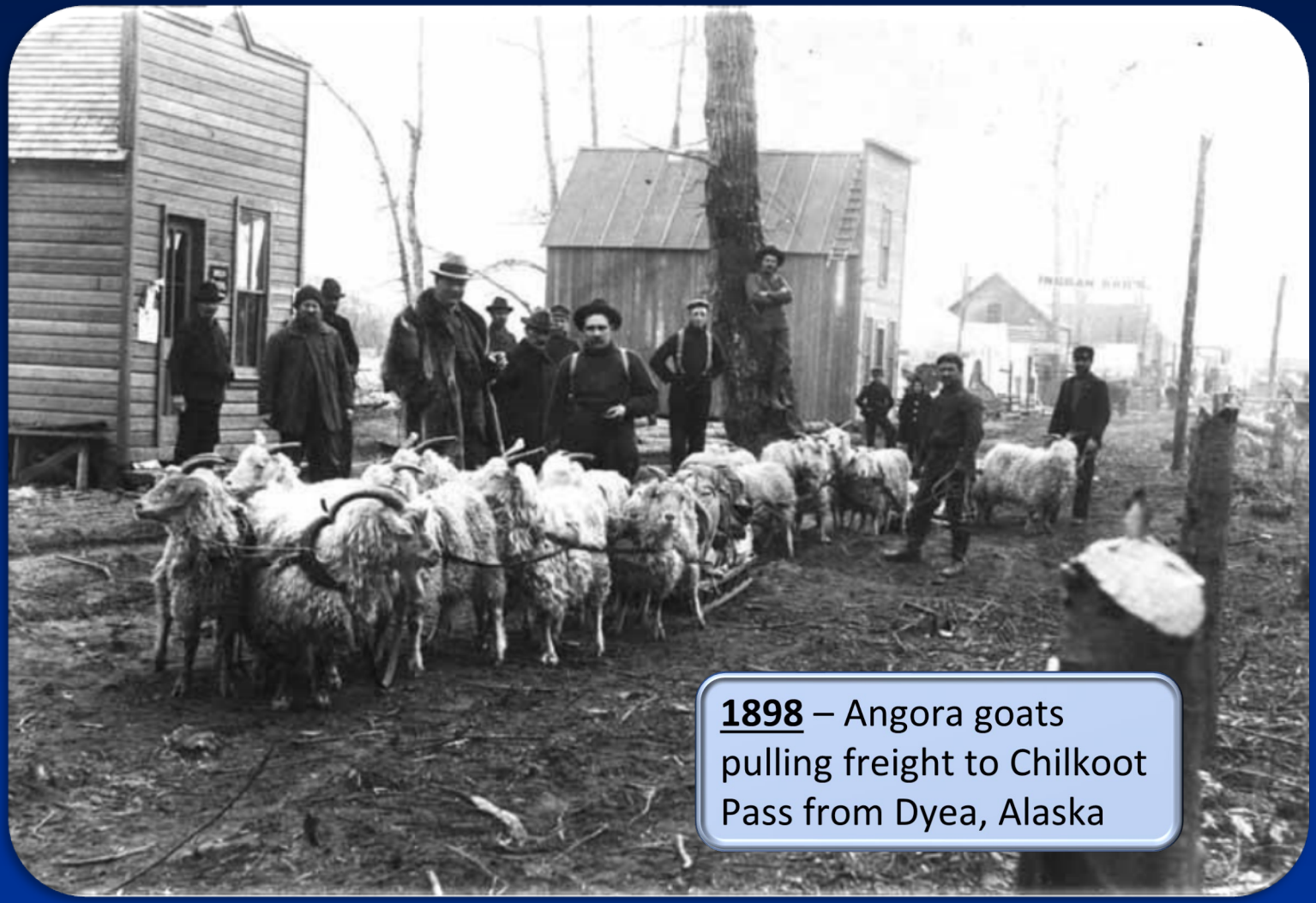
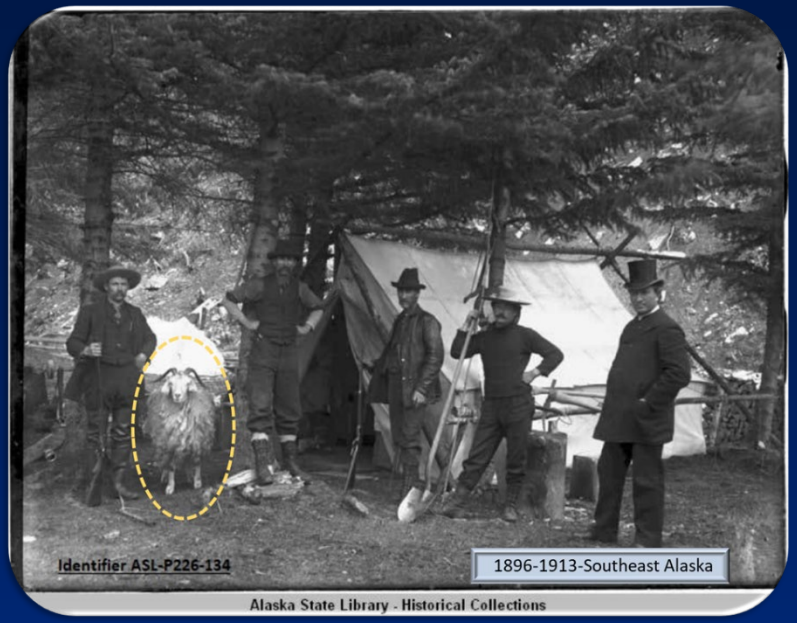
Summary

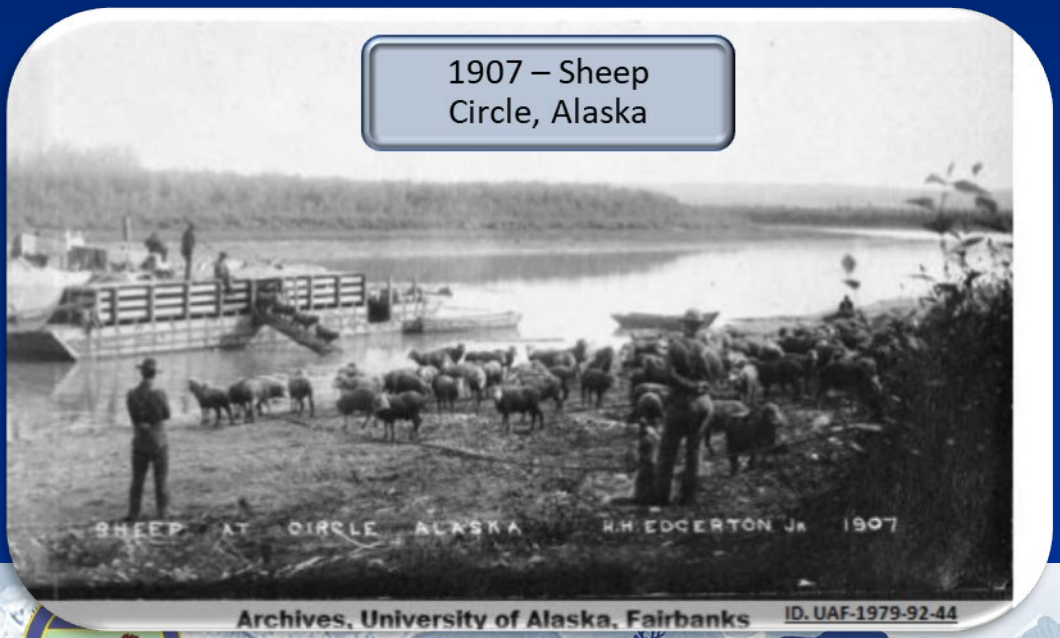
1. Dall's sheep populations potential exposures to domestic sheep and goats since the late 1800's
2. The same strain of *Mycoplasma ovipneumoniae* has been present in Alaska Dall's sheep at minimum since 2004 and caribou herds since 2007, suggesting an enzootic strain
3. No decrease in risk of introduction, exposure or adverse health impacts from other strains of M. *ovipneumoniae* is expected or implied

Outline

- Domestic animals in Dall's sheep ranges
- Respiratory Pathogen Discovery in Alaska Wild and Domestic Ungulates
- Hunter-harvest surveillance, retrospective studies targeting *M. ovi*
- Test concordance study
- Strain-typing
- Ongoing and Future work


1898 – Angora goats pulling freight to Chilkoot Pass from Dyea, Alaska

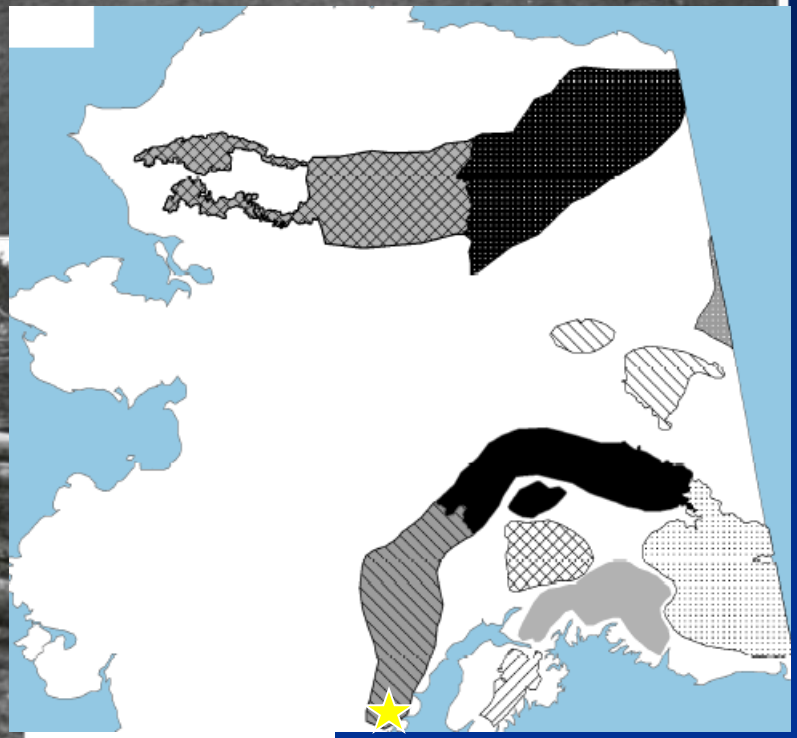




June 18, 1912
Fairbanks Daily News Miner

Port Alsworth, Lake Clark 1940's

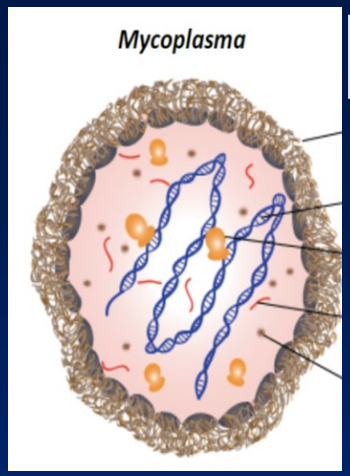
Direct contact as a potential for pathogen exposure…



This photo was taken in back of Babe Alsworth's hanger at Port Alsworth in the 1940s. Babe had goats from about 1945

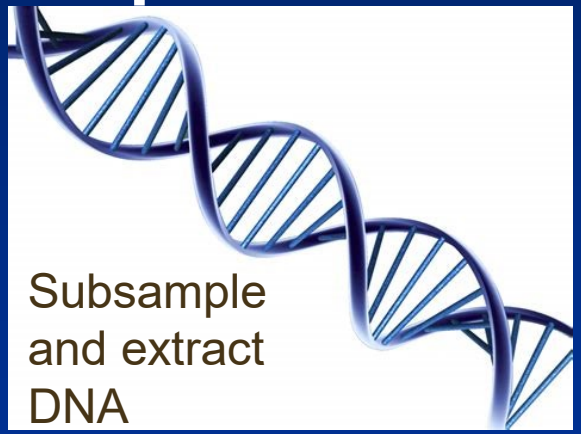

SUZUKI
40

Mycoplasma Detection



Look at DNA

Polymerase chain reaction (amplifies DNA) for mycoplasma

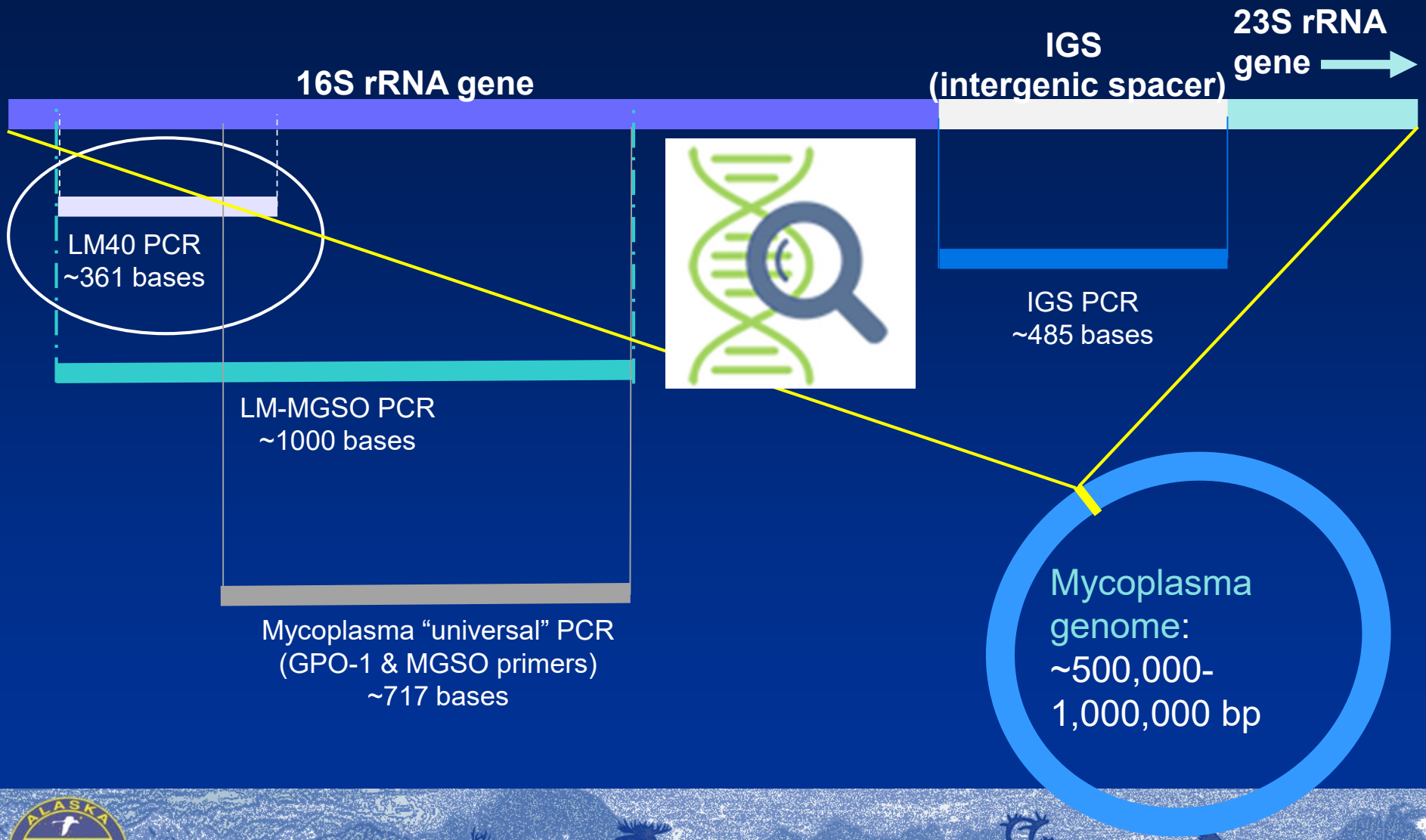


Subsample and extract DNA

Sequence DNA and compare to known sequences in GenBank

The detection of a specific species means the DNA sequence obtained from the sample matches the sequence of the known species with ≥97% similarity

Mycoplasma PCR Primers



Sampling Effort 2004-2019

Type Sampled	Species	Number of animals	Number of samples
Wildlife live capture/release	Dall's sheep, caribou, moose, mountain goats, muskox, wood bison	3703	4061 nasal swabs 261 lung samples
Hunter harvested or found dead	Above species + plains bison, Sitka black tailed deer		
Captive/zoo ungulates	Various		
Domestic animals	Sheep and goats	656	

Dall's Sheep Populations

Dall's Sheep Populations

Tested

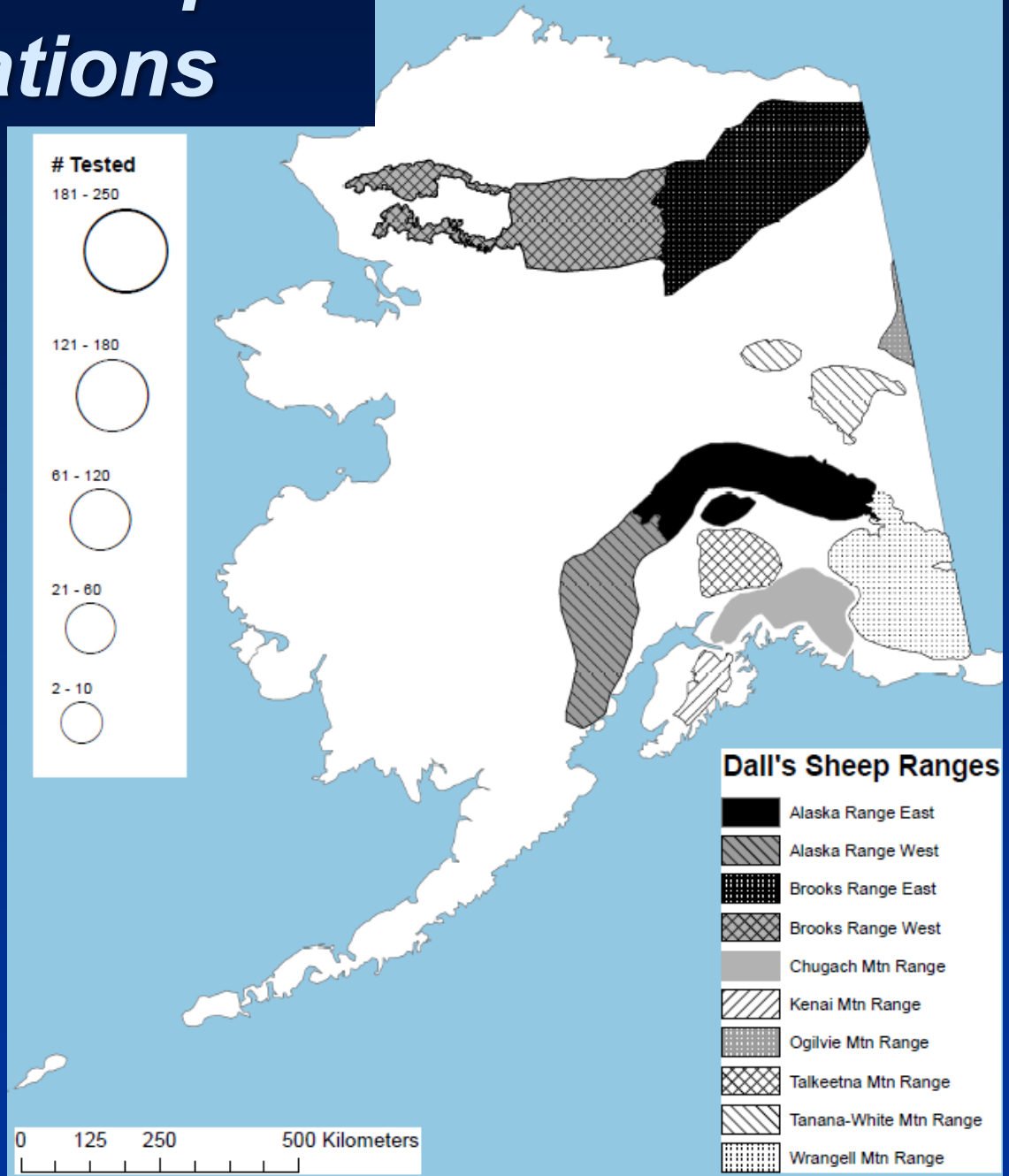
181-250

121-180

61-120

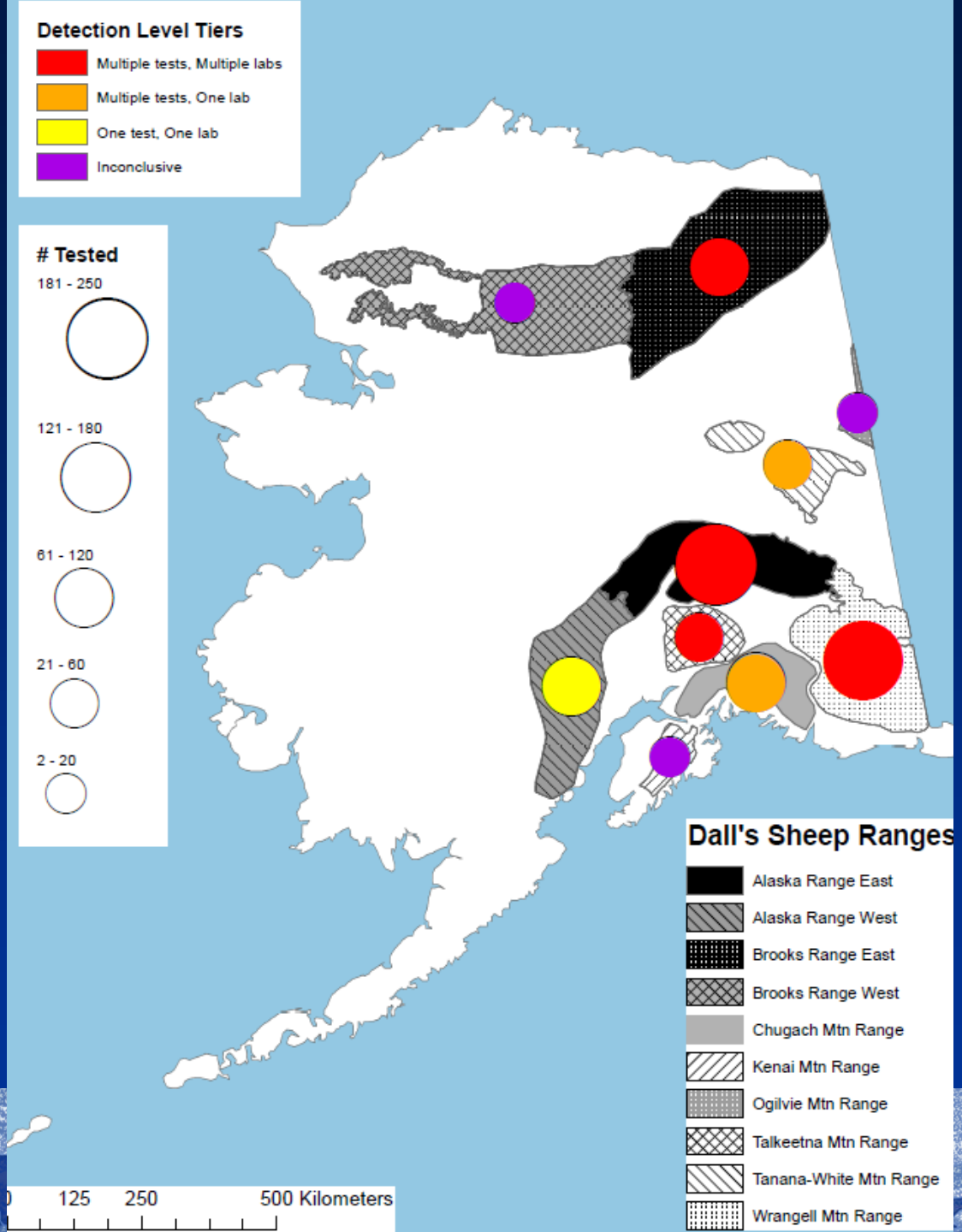
21-60

2-10



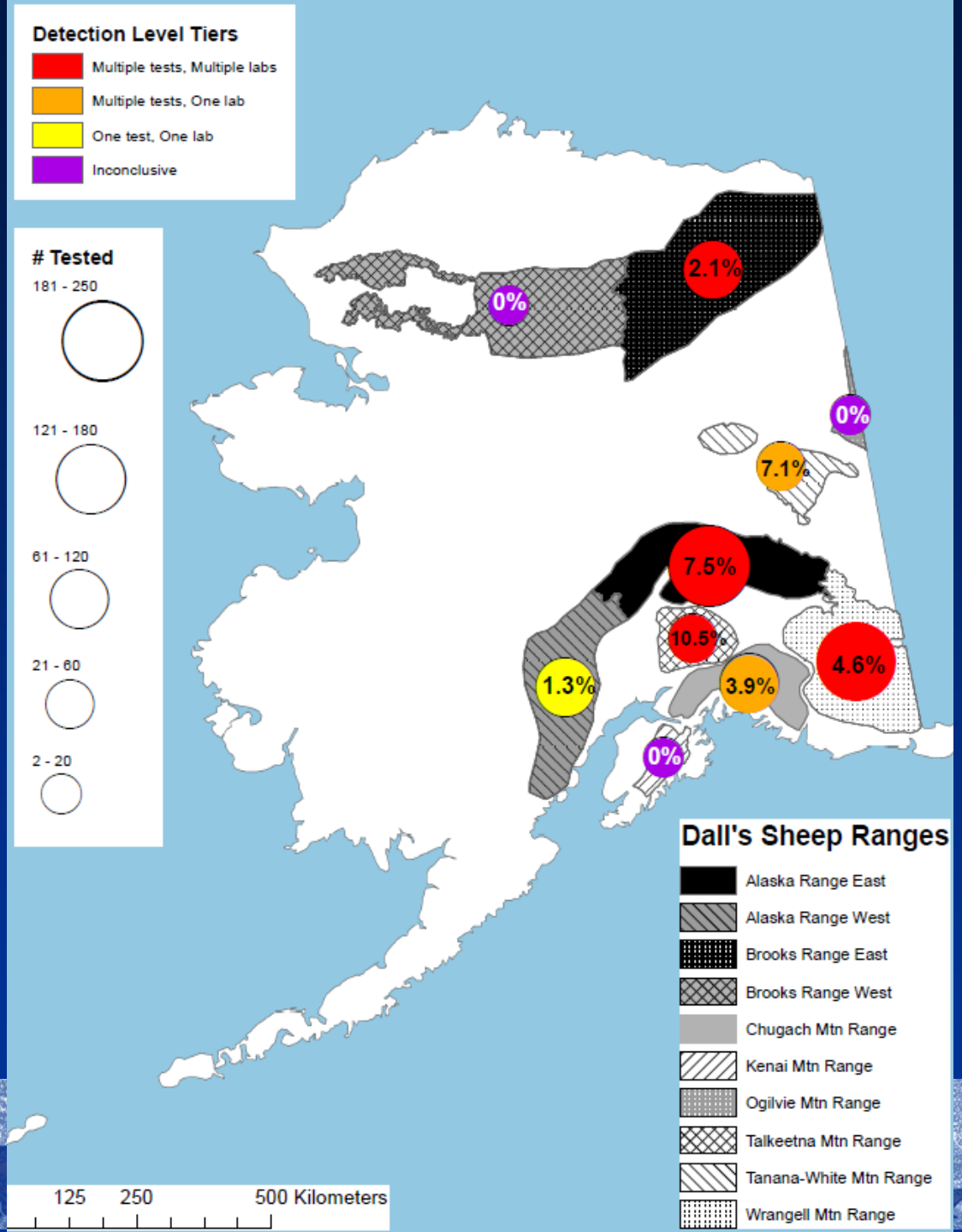
M. ovi detections in Dall's sheep 2004-2019

Red
Orange
Yellow
Purple

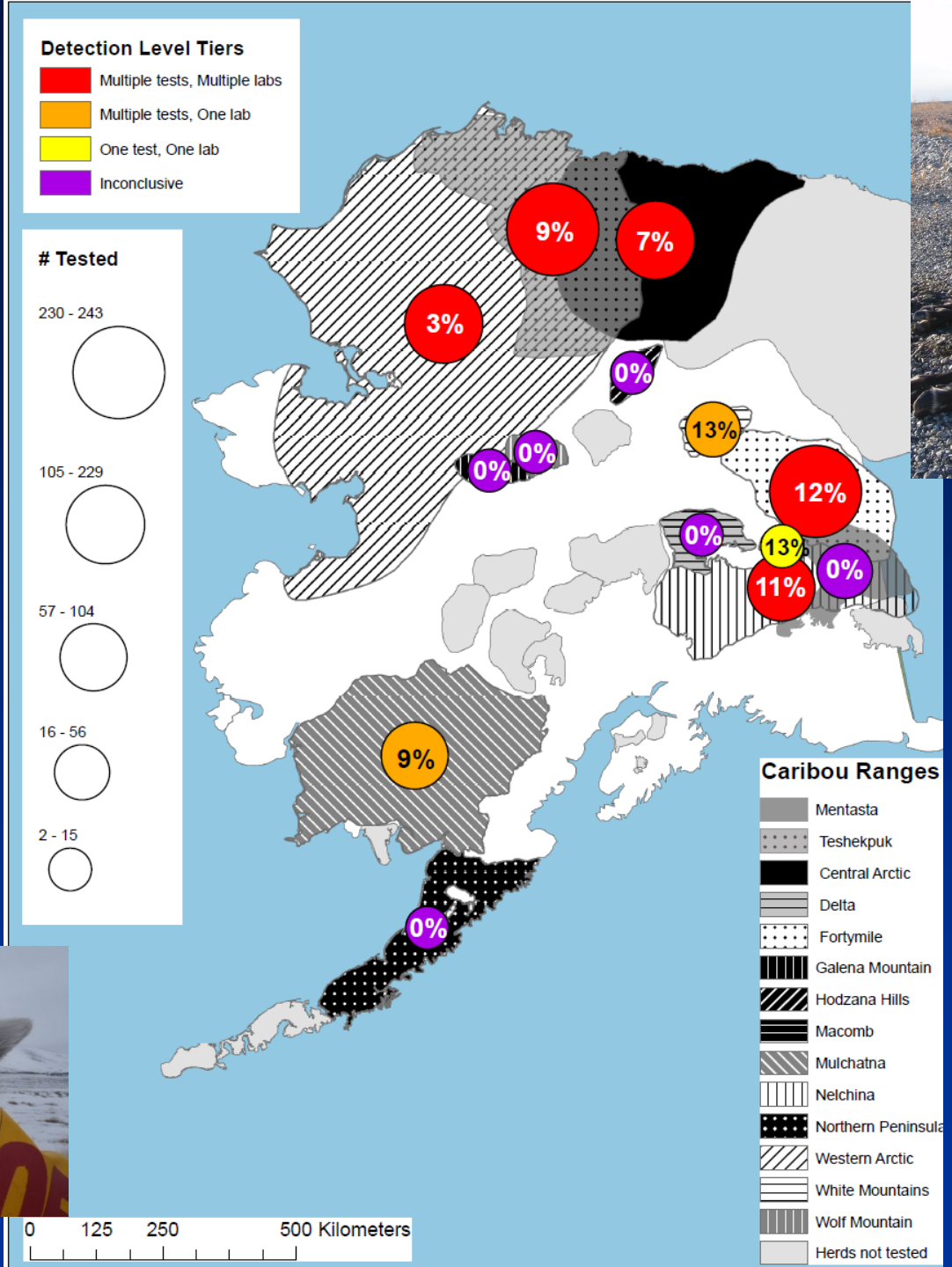


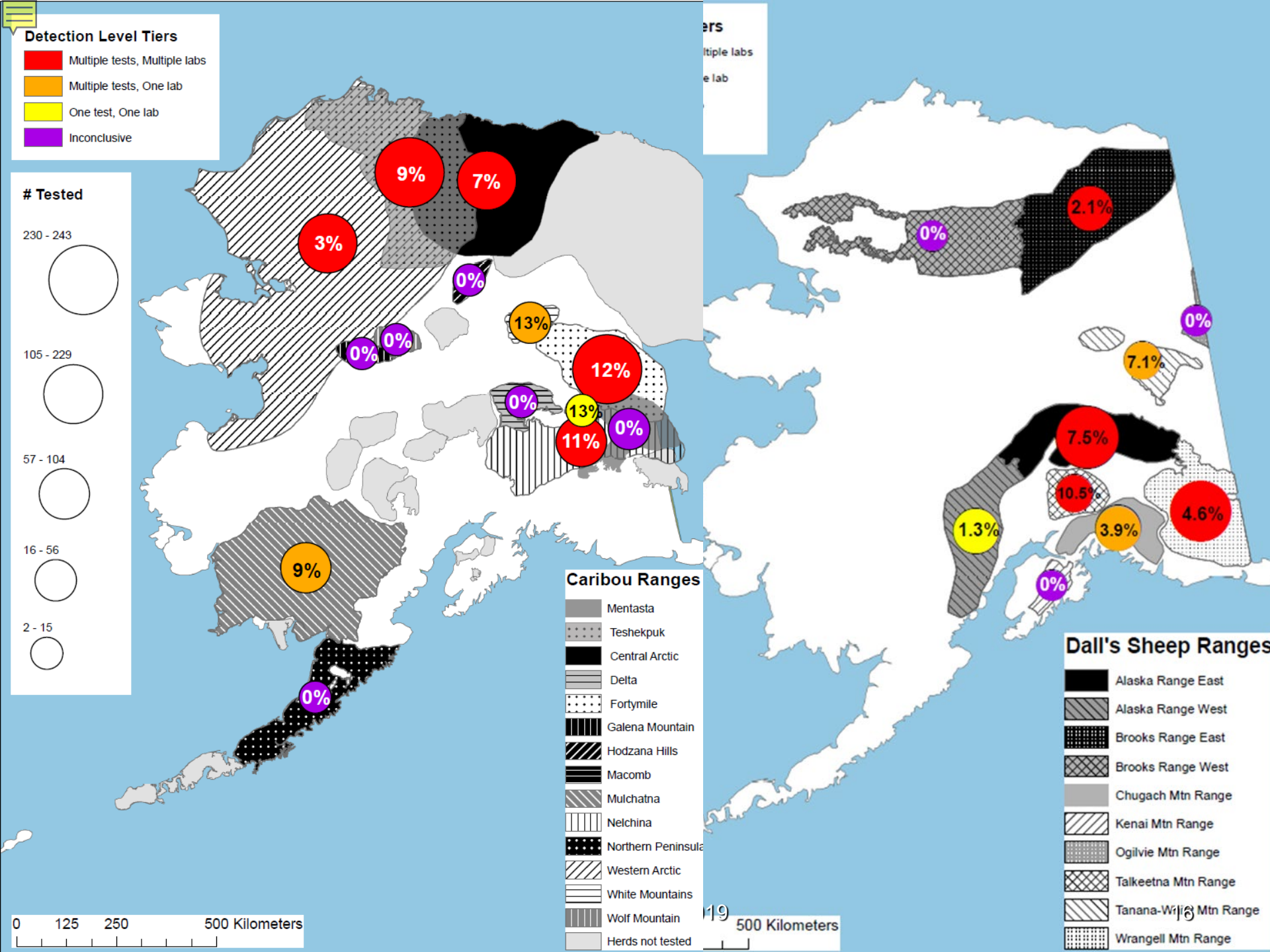
M. ovi detections in Dall's sheep

% detection as a proxy prevalence, Non-random



Caribou % detection All sources 2007-2019

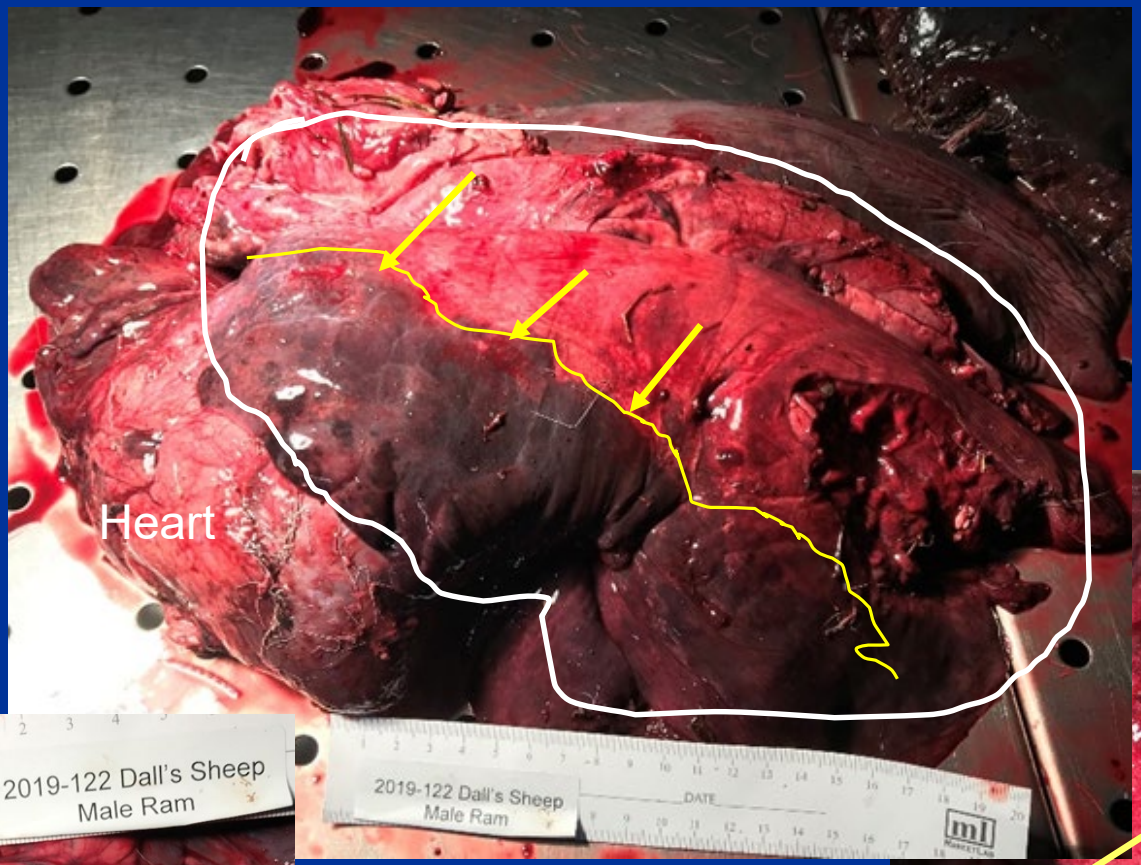


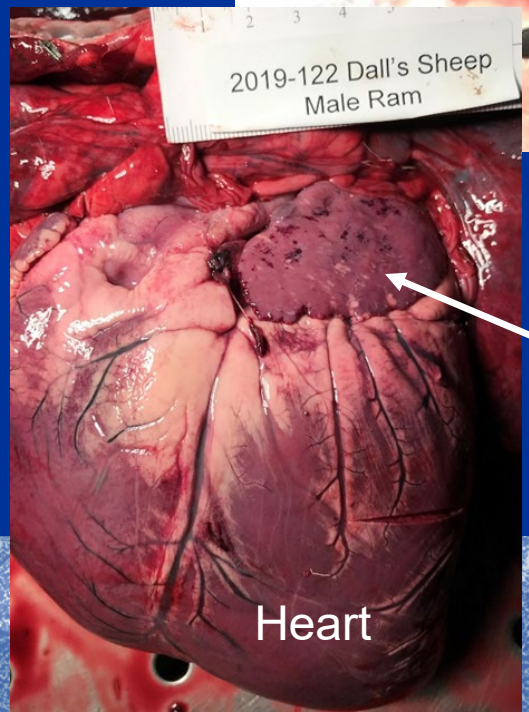

Detection Level Tiers
Multiple tests, Multiple labs
Multiple tests, One lab
One test, One lab
Inconclusive
Tested
230 - 243
105 - 229
57 - 104
16 - 56
2 - 15
9%
7%
3%
0%
0%
0%
13%
12%
0%
13%
11%
0%
9%
0%
2.1%
0%
0%
7.1%
7.5%
10.5%
4.6%
1.3%
3.9%
0%
Caribou Ranges
Mentasta
Teshekpuk
Central Arctic
Delta
Fortymile
Galena Mountain
Hodzana Hills
Macomb
Mulchatna
Nelchina
Northern Peninsula
Western Arctic
White Mountains
Wolf Mountain
Herds not tested
Dall's Sheep Ranges
Alaska Range East
Alaska Range West
Brooks Range East
Brooks Range West
Chugach Mtn Range
Kenai Mtn Range
Ogilvie Mtn Range
Talkeetna Mtn Range
Tanana-White Mtn Range
Wrangell Mtn Range
0 125 250 500 Kilometers
500 Kilometers

Late July 2019- 8yo Ram

Photo: Jeff Wells

Ram
Initial gross exam

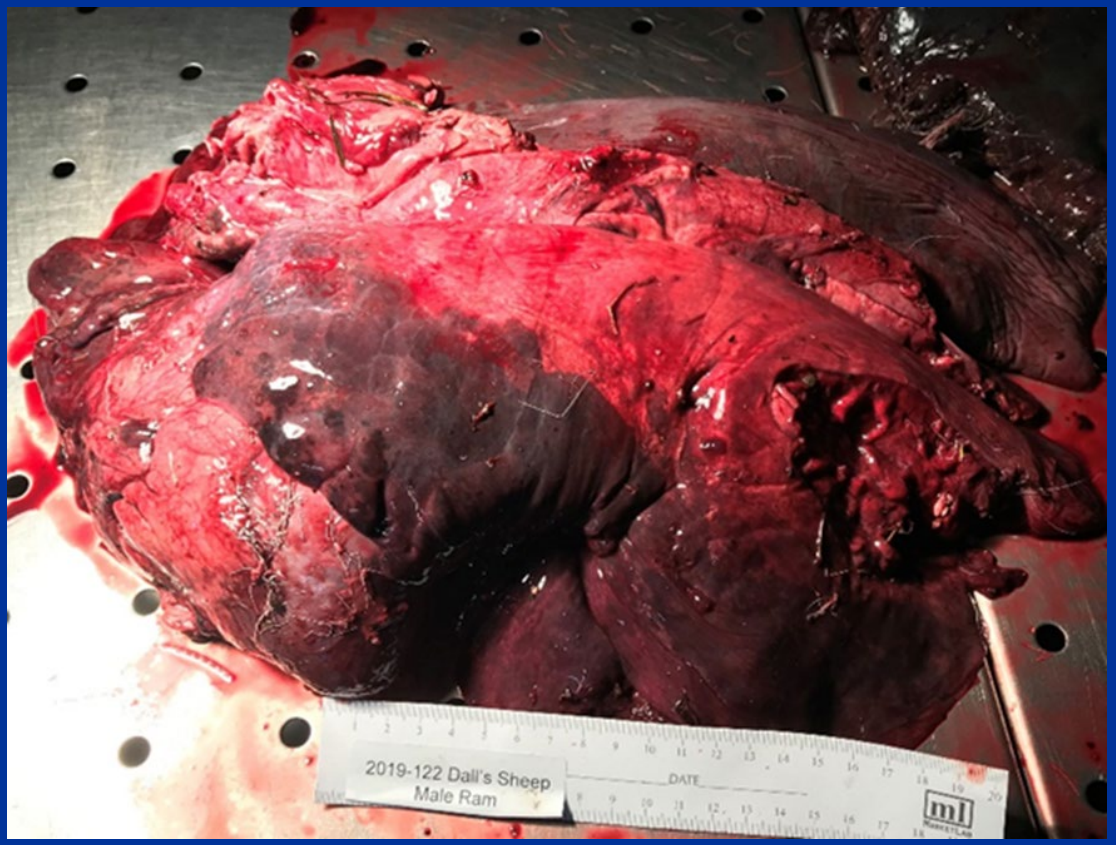




Lung consolidation

Epicardial hemorrhages

Ram
Dx: Severe subacute necrotizing bacterial bronchopneumonia



M. ovipneumoniae detected (PCR@WADDL, CVDL, WSVL, USDA)

Bibersteinia trehalosi

Trueperella pyogenes

<u>Parasites-</u>

Protostrongylus stilesi

Sarcocystis

Respiratory Pathogen Surveillance – Dall's Sheep, Molecular Techniques

- Sheep necropsies 2002-2019 n=76
- Archive frozen lungs: 24 w/ lung lesions, 15 without pneumonia, 11 recent necropsies
- Embedded, fixed lung or LN n=4
- PCR
  - *M. ovipneumoniae*
  - Respiratory Syncytial virus
  - Parainfluenza-3
  - Pestivirus A & B (BVD 1 & 2)
  - Bovine Coronavirus

“Healthy” vs. “Diseased” Sheep

1. *Lungs n=50*
   - *5 + / 45 - ,* no detection in “healthy”
   - M. ovi detection rate of 14% when ‘diseased’
2. *Nasal swabs n=328*
   - 2.7% detection rate in “healthy” sheep
   - *5 +, M. ovi* detection rate of 20% when “diseased”

Alaska Range East Lamb #1

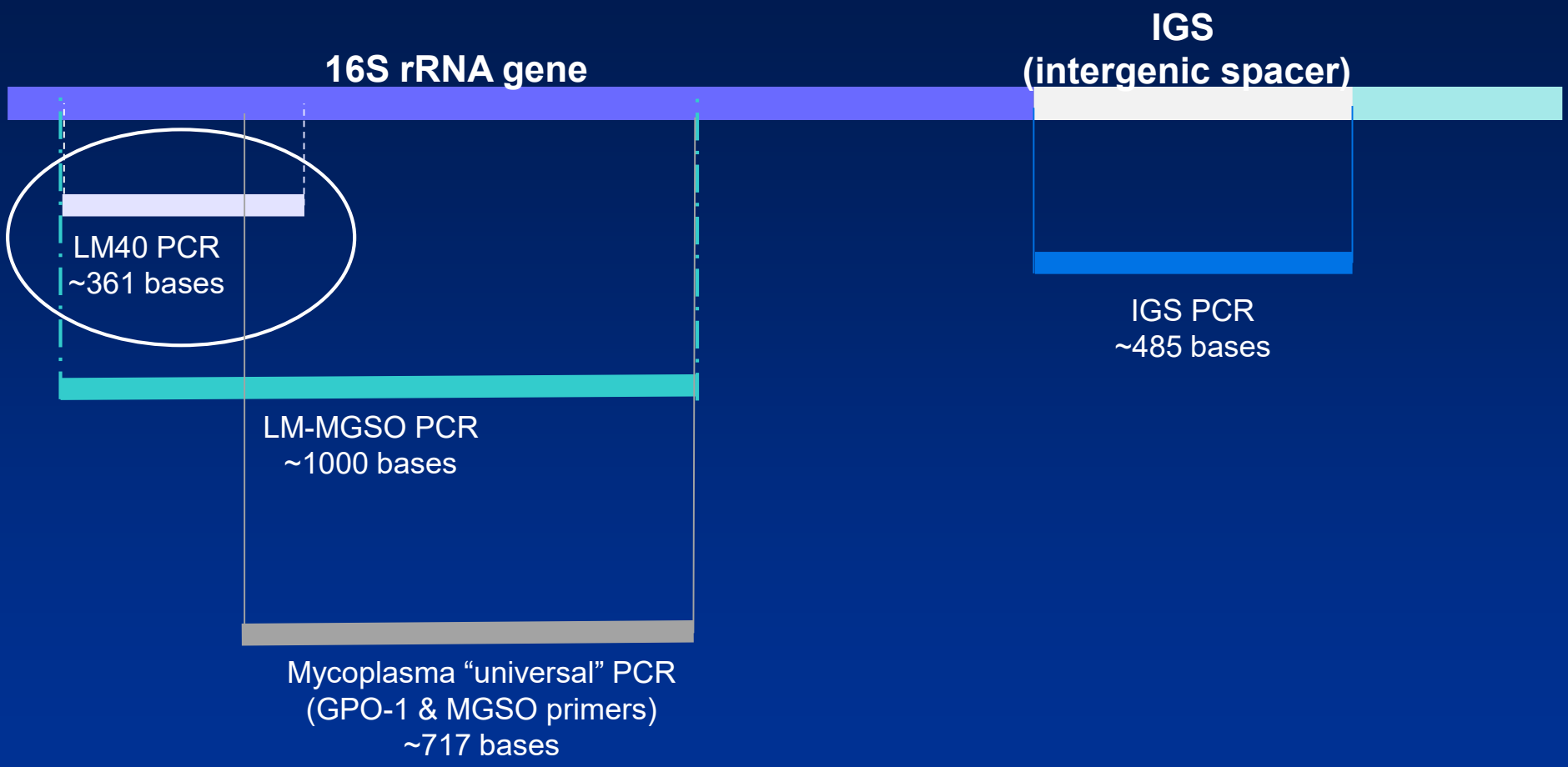
- May ‘04, Three-mile creek
- Eagle kill, Cranioventral lung consolidation
- Severe acute bacterial bronchopneumonia
  - *B. trehalosi cultured, M. ovi culture negative*
- Frozen lung M. ovi PCR Positive at WADDL & CVDL
- Fixed paraffin block M.ovi PCR Indeterminate

2004 Alaska Range East Lamb Acute polymicrobial bronchopneumonia, M.ovi +

Dall's Sheep M. ovi Serology

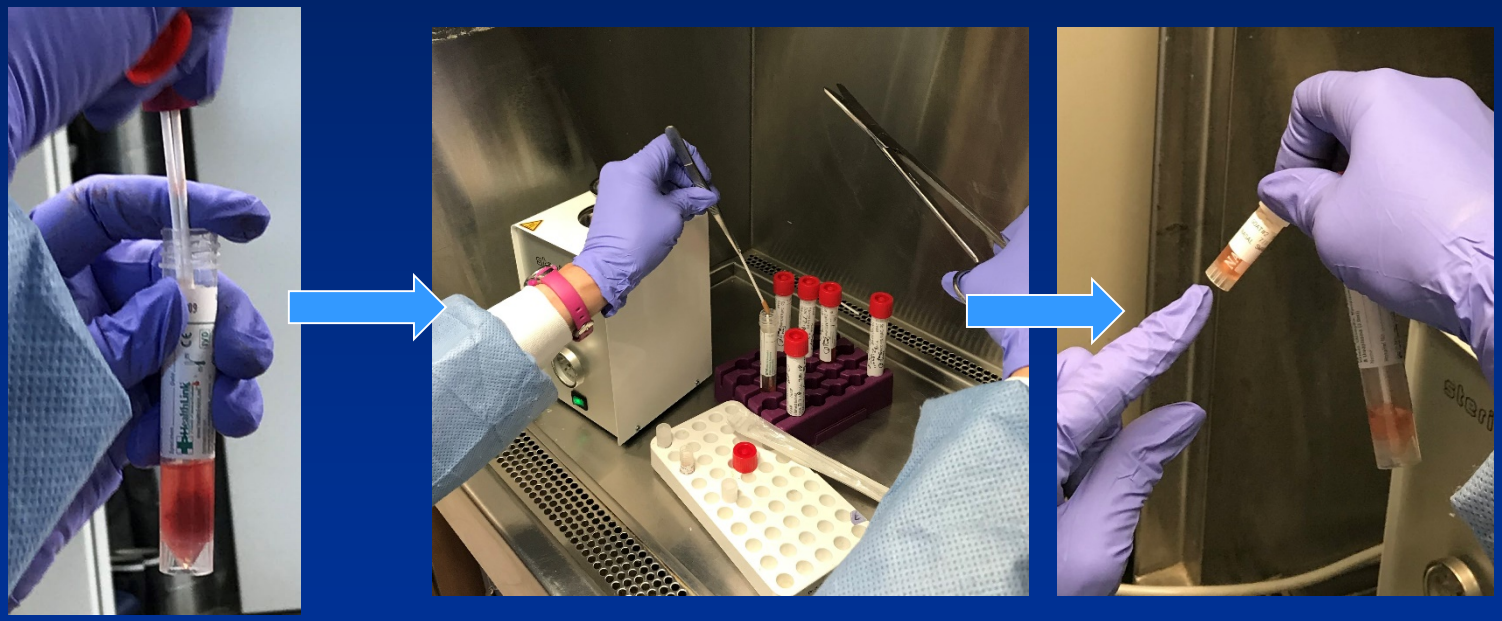
- 1979-1987 n= 253 All negative by IHA
- 2009 n=15 Chugach, 1 indeterminate by IHA
- 2009-2012 ELISA n=41 negative, 1 indeterminate, 4 positive
  - All from 2009-2010 Central Brooks range

Mycoplasma PCR Primers



Test Concordance Study

- Nasal swabs in UTM, subsampled, tested using 3 different PCR tests at 2 different laboratories
  - LM40
  - IGS
  - UM



Test Concordance Study: Species distribution

		# Detected by PCR Assay		
	# Tested	LM40	IGS	UM
Mountain Goat	53	3	0	0
Dall's Sheep	198	9	0	0
Caribou	93	17	8	9
Moose	2	0	0	0
Total	346	29	8	9

Summary of Test Concordance Comparisons

		LM40 vs IGS	LM40 vs UM	IGS vs UM
Overall Concordance		93%	94%	99%
Agreement	Positive	38%	42%	82%
	Negative	96.5%	96.6%	99.6%
Quality of Agreement		Fair	Fair	Excellent

Test Concordance Study

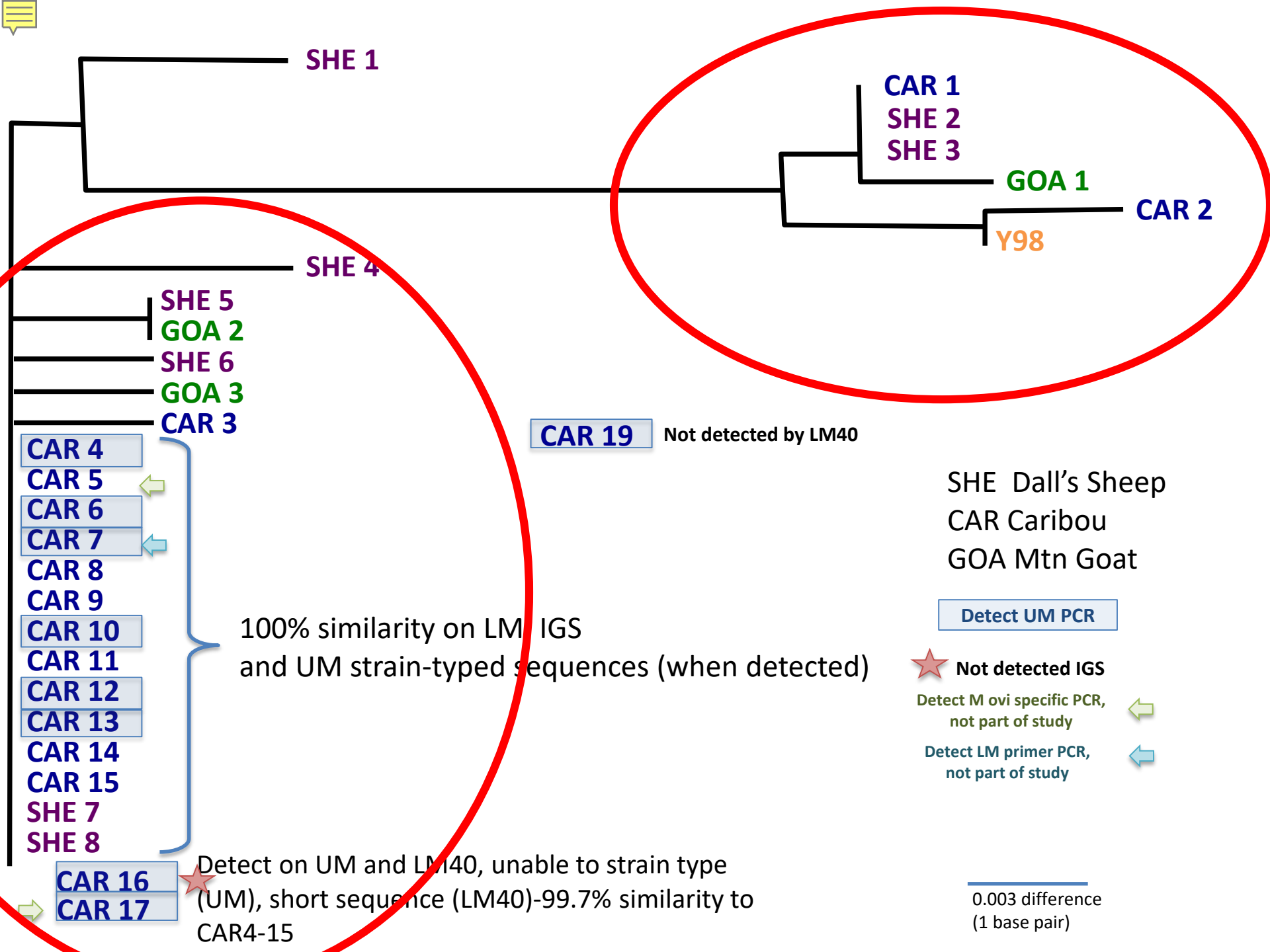
- More detections were found using the LM40 PCR than either the IGS or the Universal Mycoplasma PCR
- IGS and Universal Mycoplasma had excellent agreement

Test Concordance Follow up

- Results used to select samples for further testing
  - UM M. ovi detections were always positive in the M. ovi specific rtPCR
- WSVL detected M. ovi on LM in a sample negative at WADDL on UM & IGS at USDA but positive on LM40
- Facilitated the sequencing of M. ovi distant populations and archived tissues

Concordance Study Sequences

- LM40 and IGS detections only have detected sequences from assay region (LM or IGS)
- UM detections were strain-typed (4 loci) including LM and IGS
  - 7 of 9 caribou had the same strain type (100% identity, all loci)
  - 2 of 9 caribou unable to amplify all loci.
    - Loci amplified had 100% identity
      - 1 caribou has LM sequence
      - 1 caribou has IGS sequence


SHE 1
CAR 1
SHE 2
SHE 3
GOA 1
CAR 2
Y98
SHE 4
SHE 5
GOA 2
SHE 6
GOA 3
CAR 3
CAR 4
CAR 5
CAR 6
CAR 7
CAR 8
CAR 9
CAR 10
CAR 11
CAR 12
CAR 13
CAR 14
CAR 15
SHE 7
SHE 8
CAR 16
CAR 17
CAR 19
Not detected by LM40
100% similarity on LM IGS
and UM strain-typed sequences (when detected)
Detect on UM and LM40, unable to strain type (UM), short sequence (LM40)-99.7% similarity to CAR4-15
SHE Dall's Sheep
CAR Caribou
GOA Mtn Goat
Detect UM PCR
Not detected IGS
Detect M ovi specific PCR, not part of study
Detect LM primer PCR, not part of study
0.003 difference
(1 base pair)

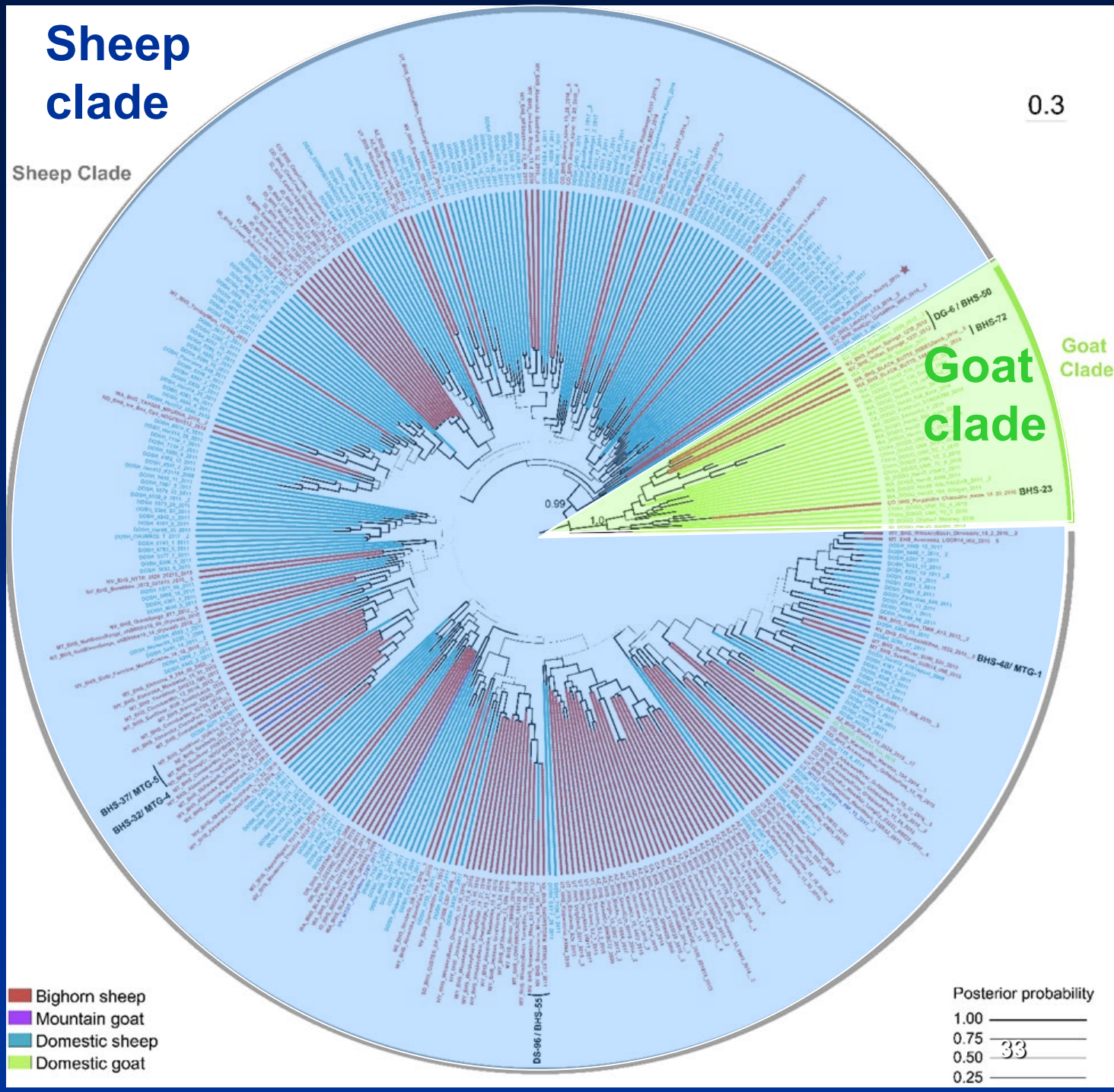
Kamath et al 2019

M. ovipneumoniae consensus tree

Alaskan wildlife: Have strain typed

- 11 Dall's sheep from 3 range areas sampled from 2004-2019
- 12 caribou from 5 herds collected 2007-2019
- All but one sequence is identical
  - Differs by 1 base pair

- The sequences are most closely related to those in the sheep clade



Summary

- Archived samples of lungs from 2004 Alaska Range Dall's sheep and 2007 arctic caribou reveal essentially the same strain type as contemporary Dall's sheep and caribou across AK
- Geographic and species distribution consistent with an enzootic organism in caribou and Dall's sheep

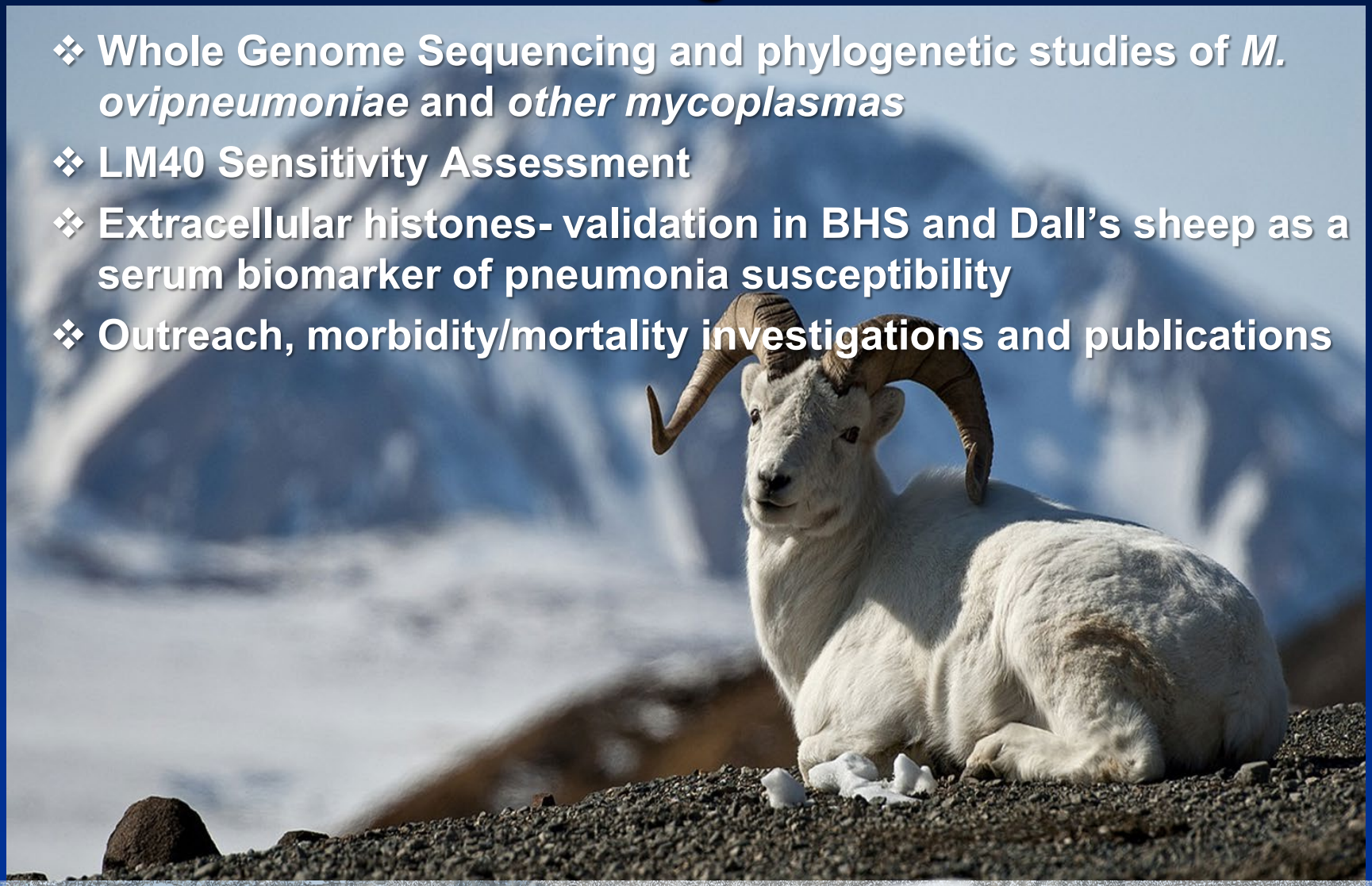
Summary

- Presence of an enzootic strain does not suggest any decrease in vulnerability or risk to Alaska wild ungulates from other M. ovi strains, respiratory pathogens or an outbreak of disease of the enzootic strain under additional stressor
- There is a lot more that we don't know than what we do know




In Progress

- Whole Genome Sequencing and phylogenetic studies of *M. ovipneumoniae* and *other mycoplasmas*
- LM40 Sensitivity Assessment
- Extracellular histones- validation in BHS and Dall's sheep as a serum biomarker of pneumonia susceptibility
- Outreach, morbidity/mortality investigations and publications



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