Ungulate Respiratory Pathogens:
Alaska Research Findings

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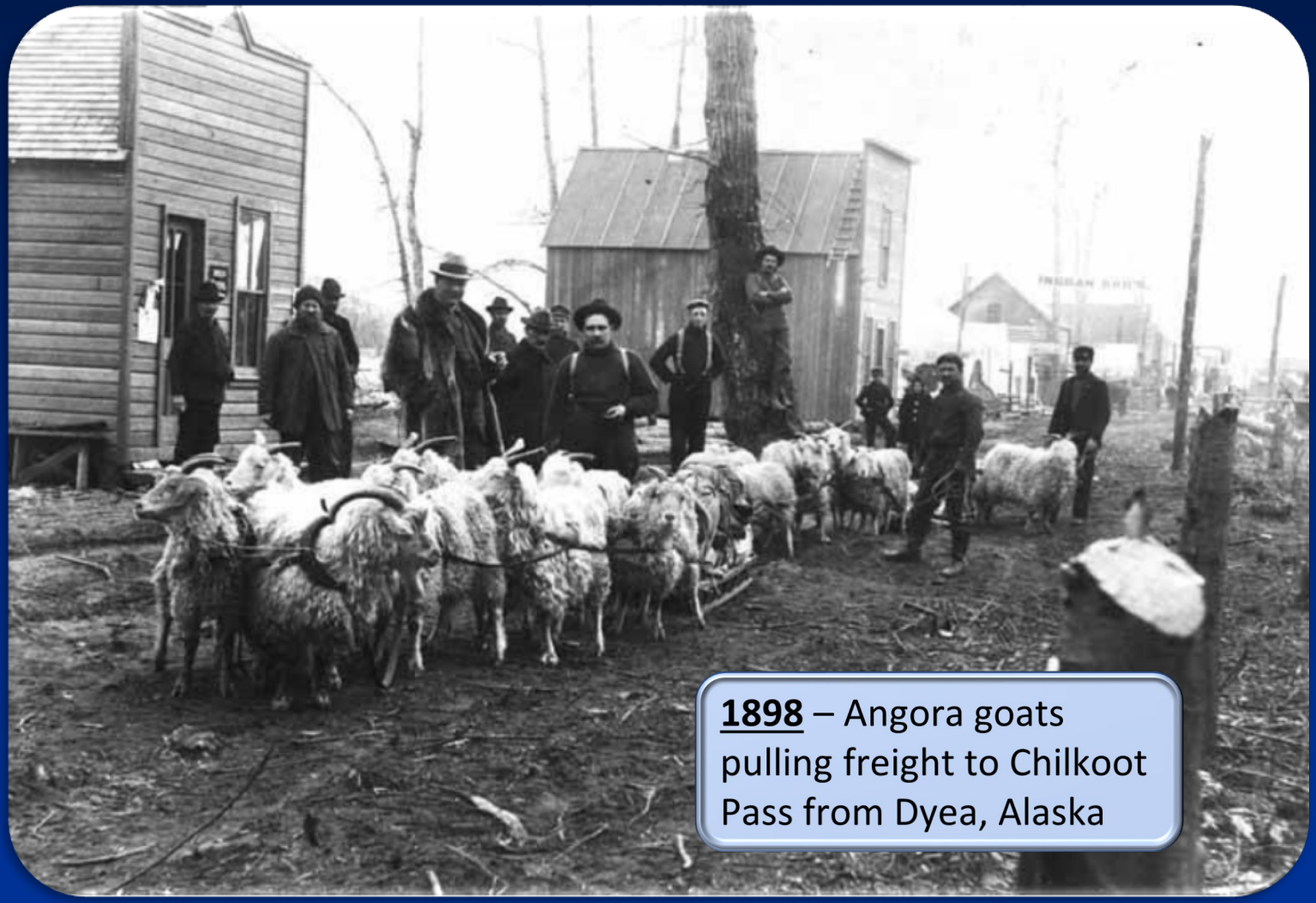
Outline

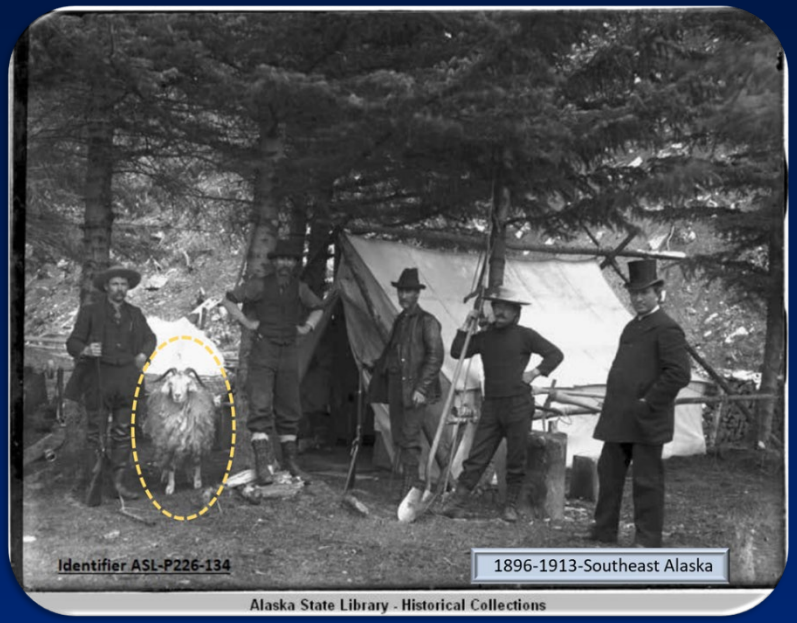
- Domestic Animal-Wildlife Interactions
- Respiratory Pathogen Discovery in Alaska Wild and Domestic Ungulates
- Wildlife and animal surveillance, retrospective studies targeting M. ovi
- Test concordance study
- Strain-typing
- Ongoing and Future work

Domestic-Wildlife Interactions

- Predation
- Habitat competition: Land, forage, water
- Pathogen issues: (> 60% are zoonotic)
  - Bovine Brucellosis: Greater Yellowstone Area
  - Bovine Tuberculosis: Michigan
  - Parasites and Tick-borne diseases:
    - Lyme Disease, Anaplasmosis, Babesia
    - Cattle Fever Tick, Asian Longhorn Tick
    - Moose Winter Tick
  - Rabies
  - Avian Influenza
  - Plague
  - **Respiratory Pathogens**

Historic research by J. Want





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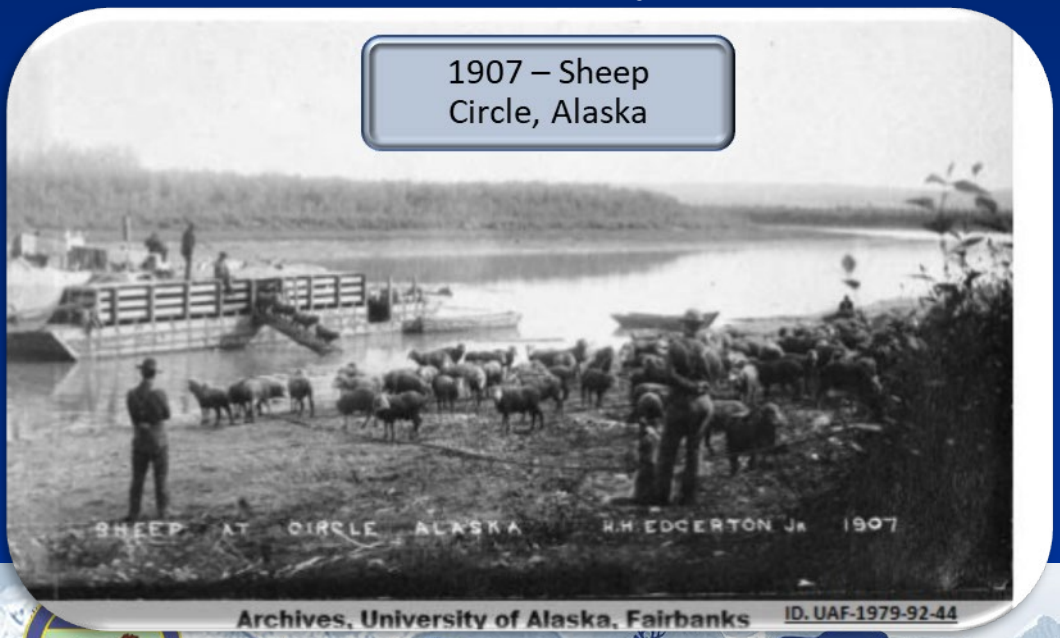
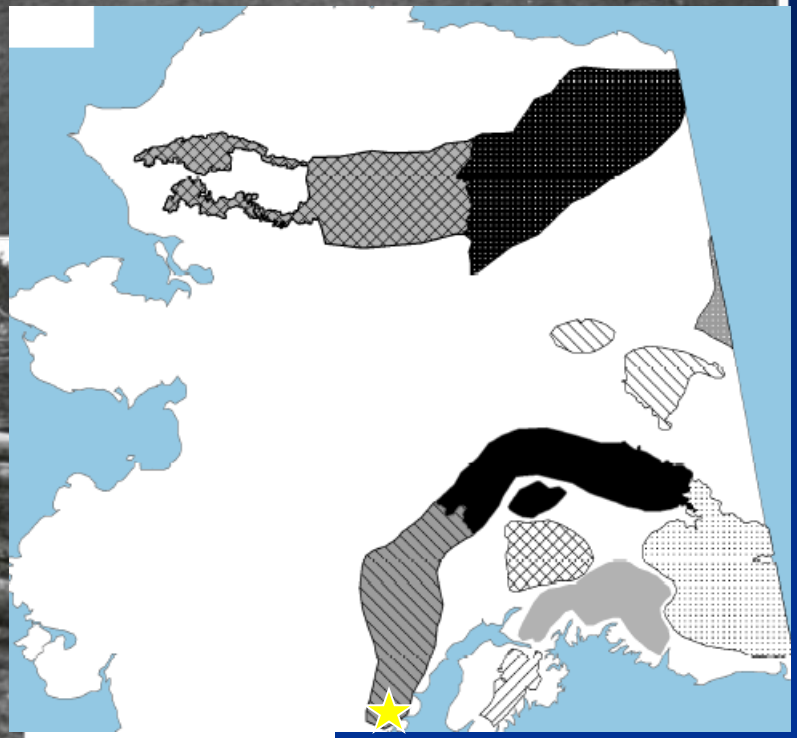


June 18, 1912
Fairbanks Daily News Miner

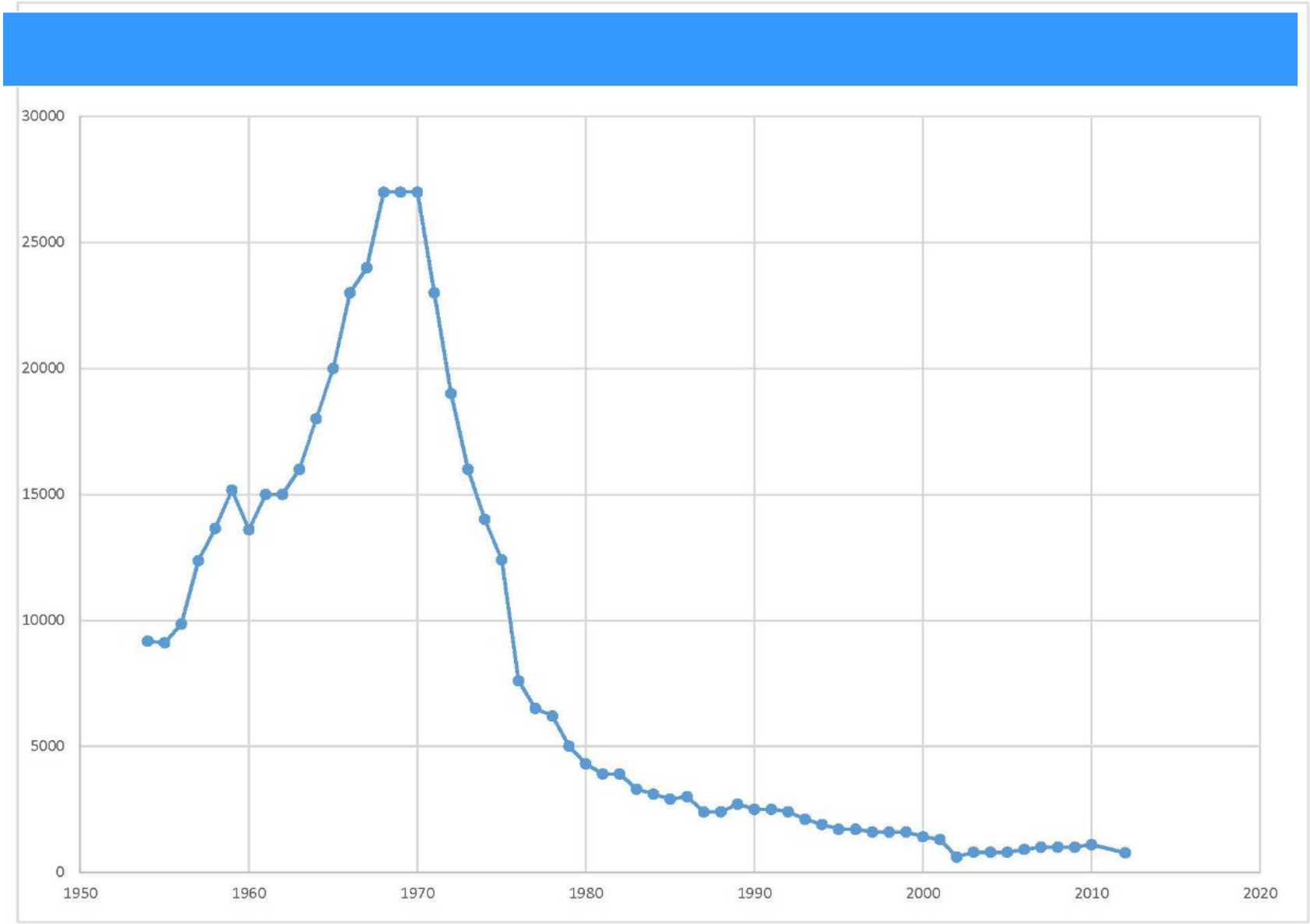
Port Alsworth, Lake Clark 1940's

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

Populations of Domestic Sheep in Alaska



Data provided by Dr. Bob Gerlach


SUZUKI
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Research Investigating Causes of, and Agents Associated with, Respiratory Disease In Domestic and Wild Ungulates

Cooperative Study Objectives

- Determine the prevalence, distribution, and health impacts of respiratory pathogens
- Understand potential sources/reservoirs with a focus on *Mycoplasma* spp. in domestic and wild ruminants
- Characterize *Mycoplasma spp.* and investigate potential roles in animal health.
  - Targeting the 'unique', unidentifiable moose mycoplasma detected in 2015

USDA ARS Agricultural Research Service

ALASKA Department of Environmental Conservation

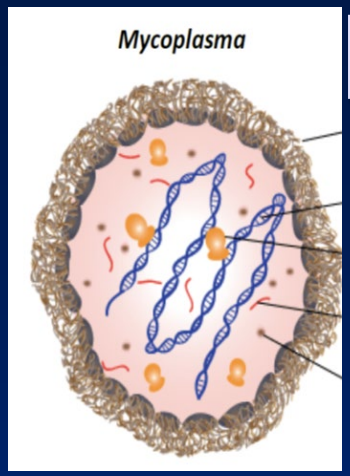
What is M. ovi?

- M. ovi is short for *Mycoplasma ovipneumoniae*
- A bacterium that can lead to pneumonia particularly in sheep, goats and other Caprinae
- Domestic sheep and goats can carry the bacterium without overt disease
- Not all strains cause sickness and additional stressors (nutritional/environmental/parasites/physiologic state) are important factors

M. ovi in wild sheep populations

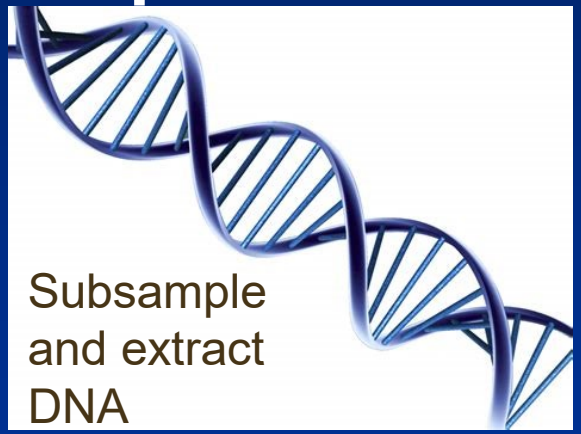
- Primary bacteria in **multifactorial** pneumonia outbreaks caused some large die-offs of bighorn sheep
  - reduced lamb survival following outbreaks
- Bighorn sheep – lower resistance to some domestic sheep pathogen exposure and suffer more severe disease when infected
  - No apparent cross immunity from previous outbreak genotypes
- Mortalities in Norway in muskoxen
- Zoo Dall's sheep exposed to domestic sheep

Mycoplasma Detection



Look at DNA

Polymerase chain reaction (amplifies DNA) for mycoplasma

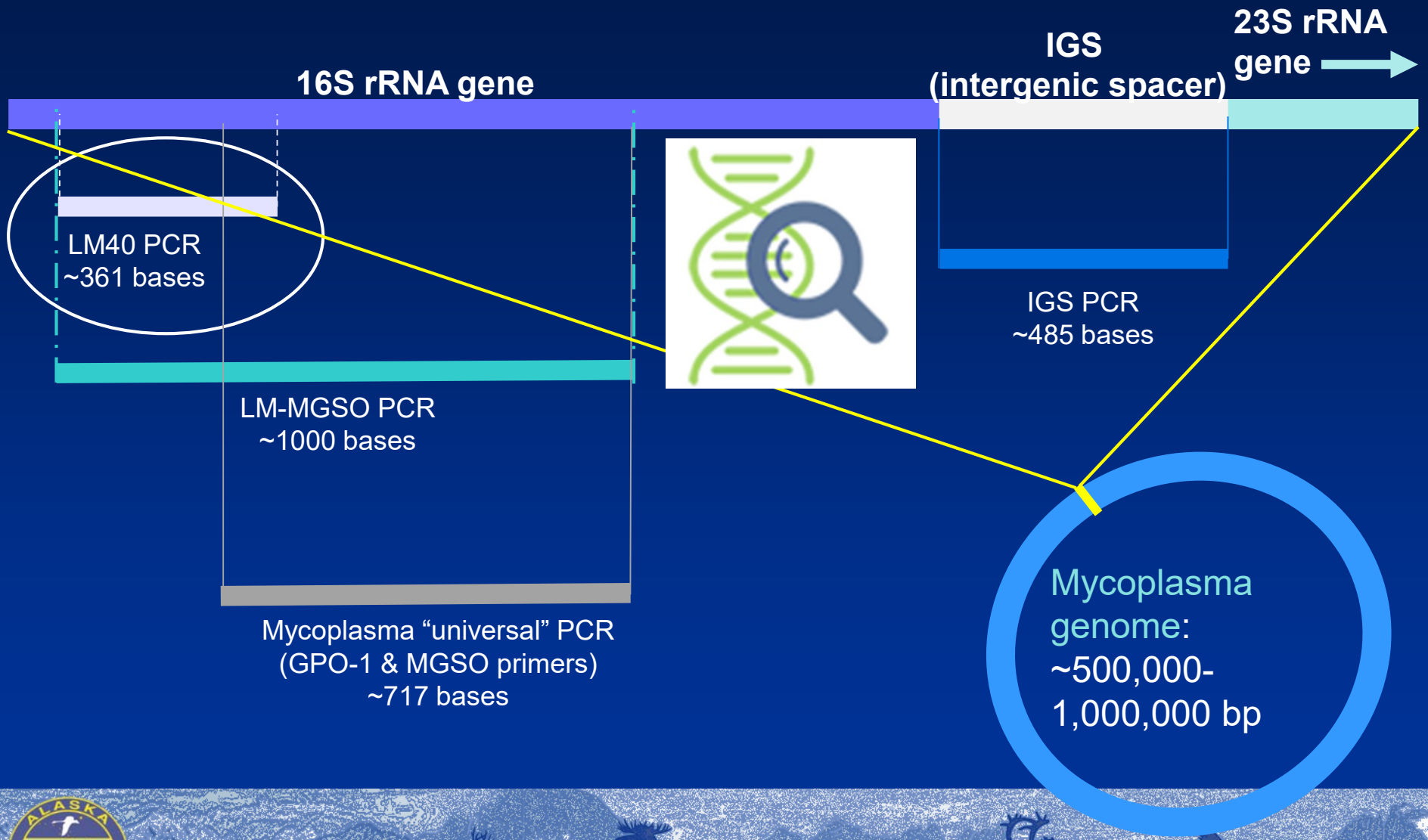


Subsample and extract DNA

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	

Results n=656 Domestic Animals

- 43 farms: 7 sheep only, 32 goats only, 4 both
- 11 farms **(26 %)** M ovi was detected
  - sheep farms 4/7 (57%), 7.6% of sheep
  - goat farms 4/32 (12%), 2.5% of goats
- Repeat sampling rarely found consistent shedding in an individual
- Some seropositive farms had no PCR detections
- Similar to other studies, smaller farms and goat farms have lower detection prevalences

ALASKA Department of Environmental Conservation

Dall's Sheep Populations

BOG March 2020

Dall's Sheep Populations

Tested

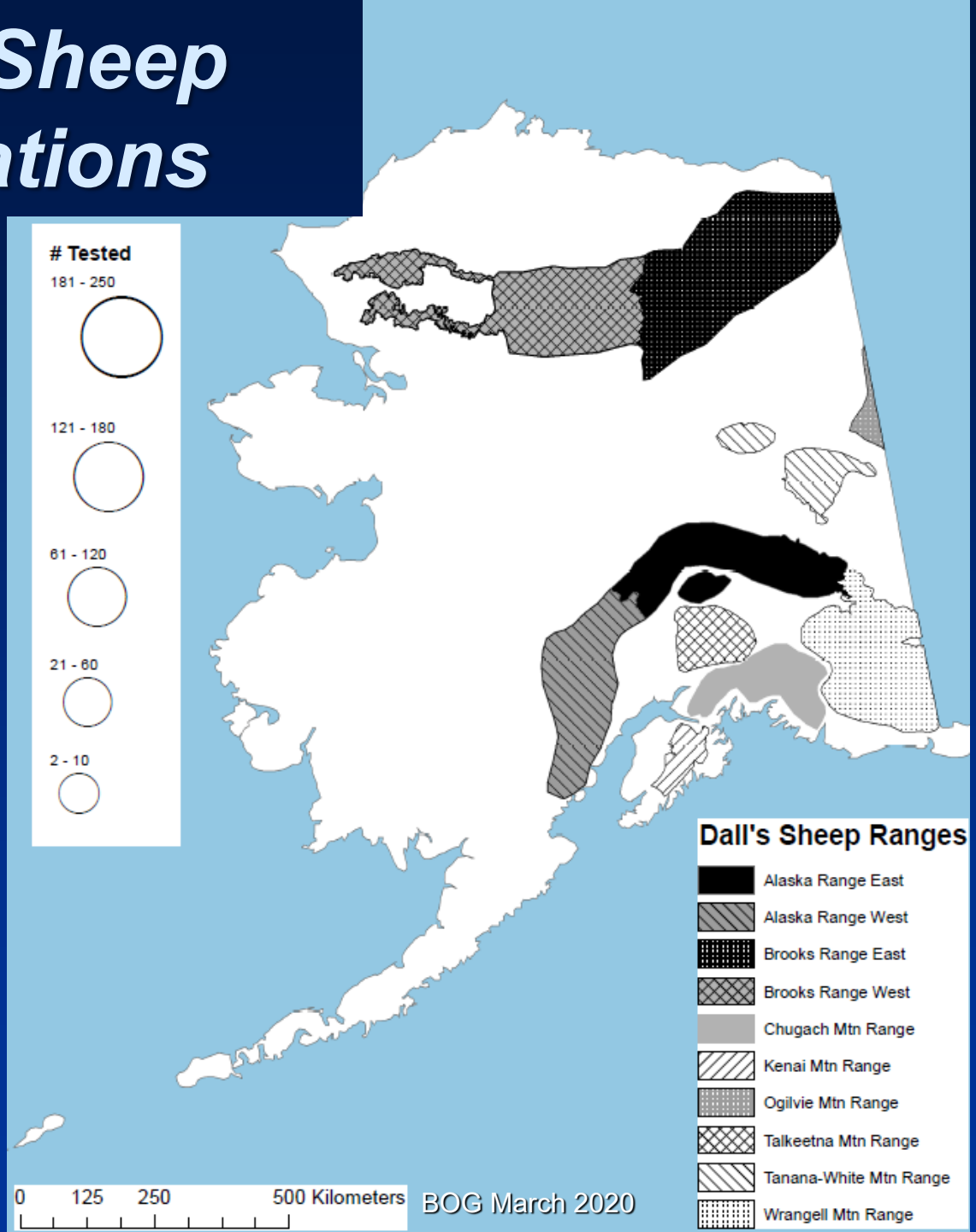
181-250

121-180

61-120

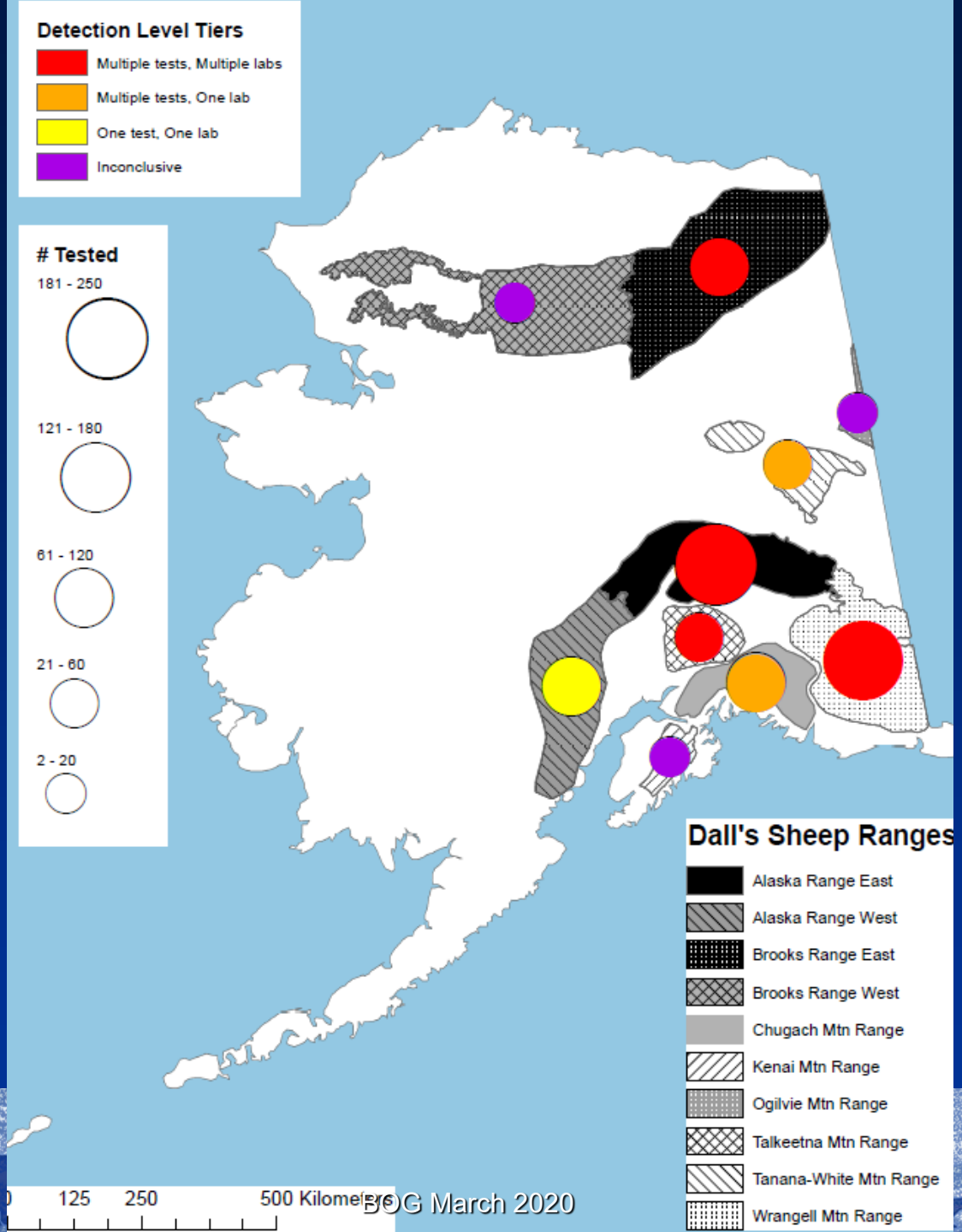
21-60

2-10



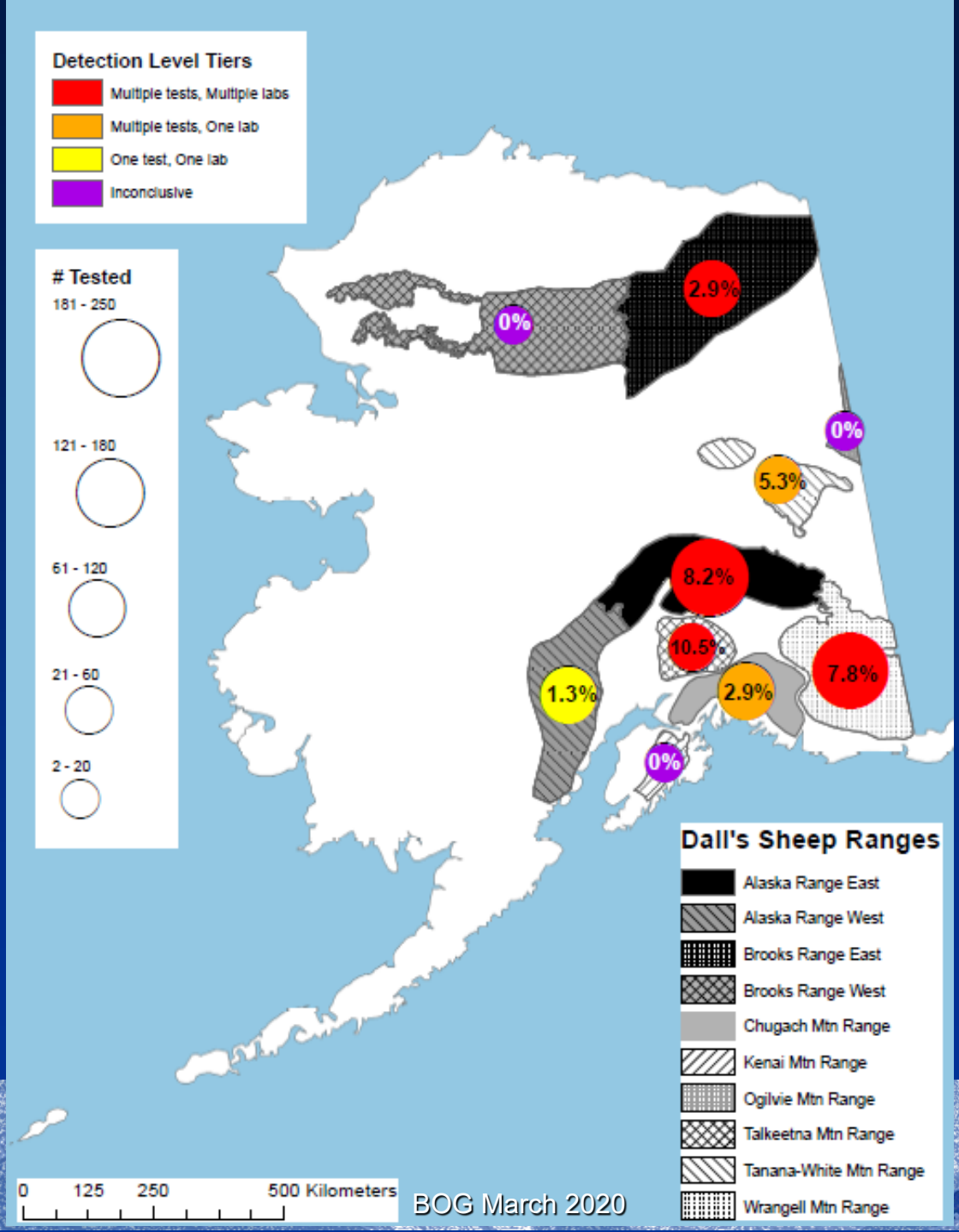
M. ovi detections in Dall's sheep 2004-2019

Red
Orange
Yellow
Purple



M. ovi detections in Dall's sheep

% detection
Non-random



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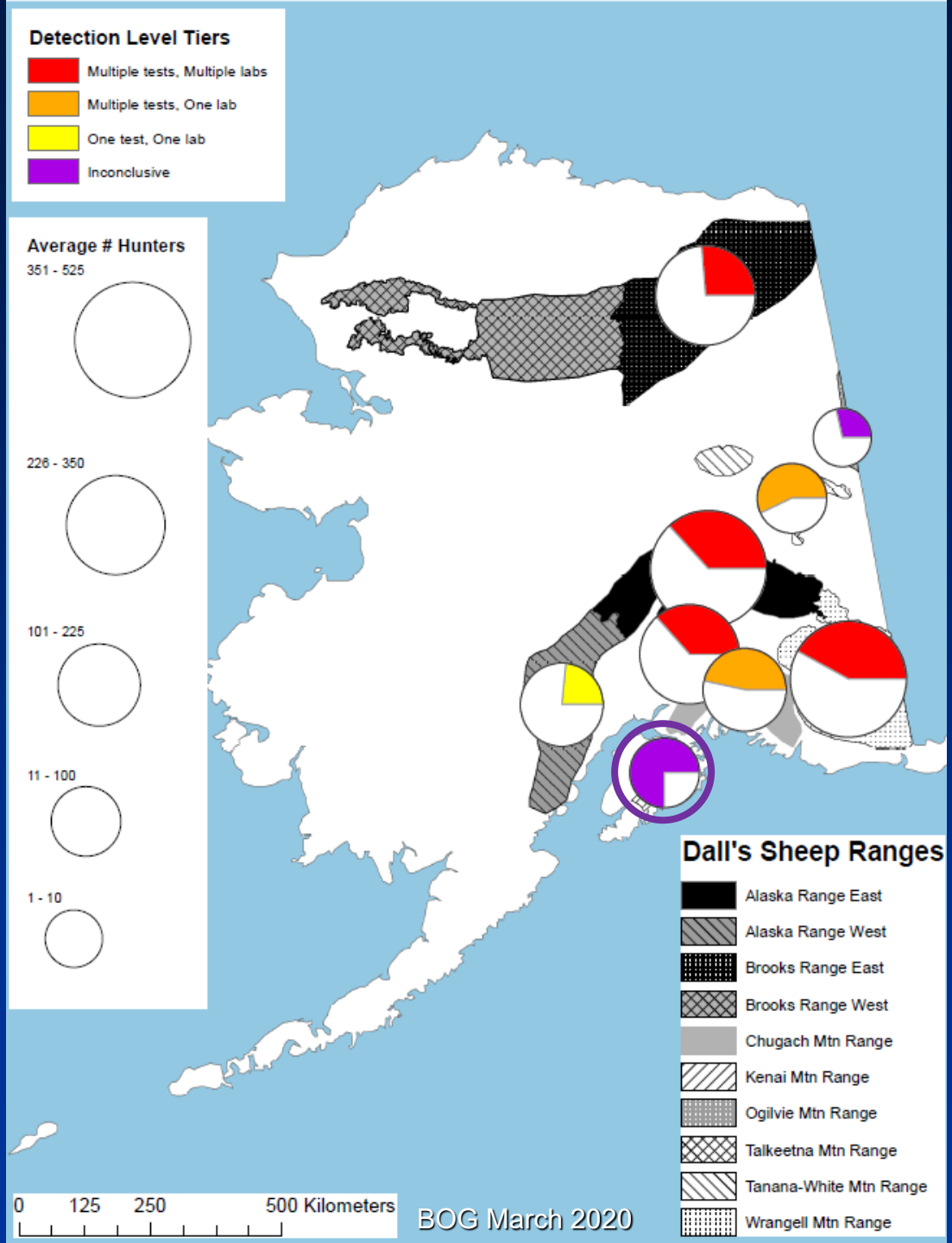
Sampling Success of Hunter Harvested Dall's Sheep FY18-FY20



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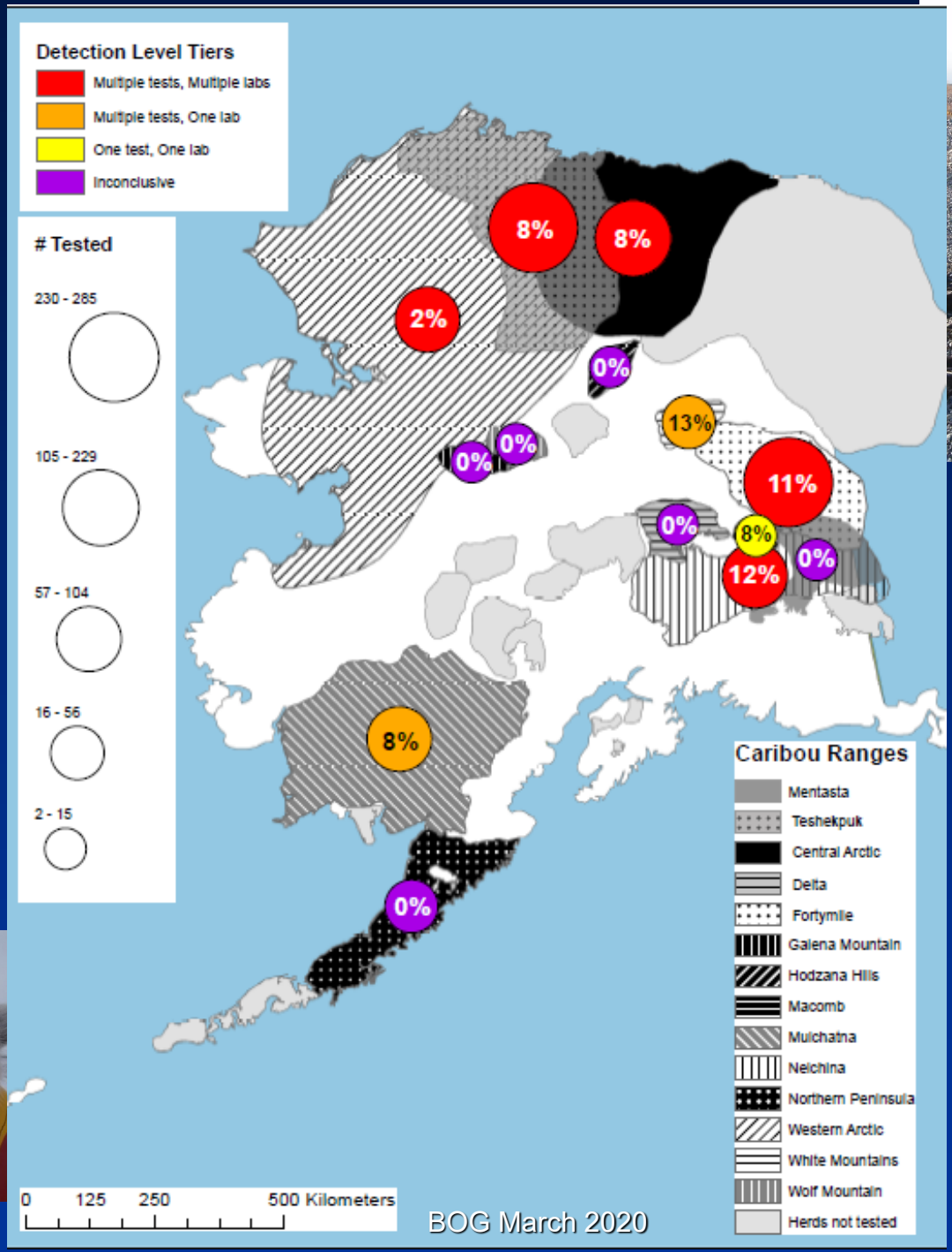
Caribou % detection All sources 2007-2019



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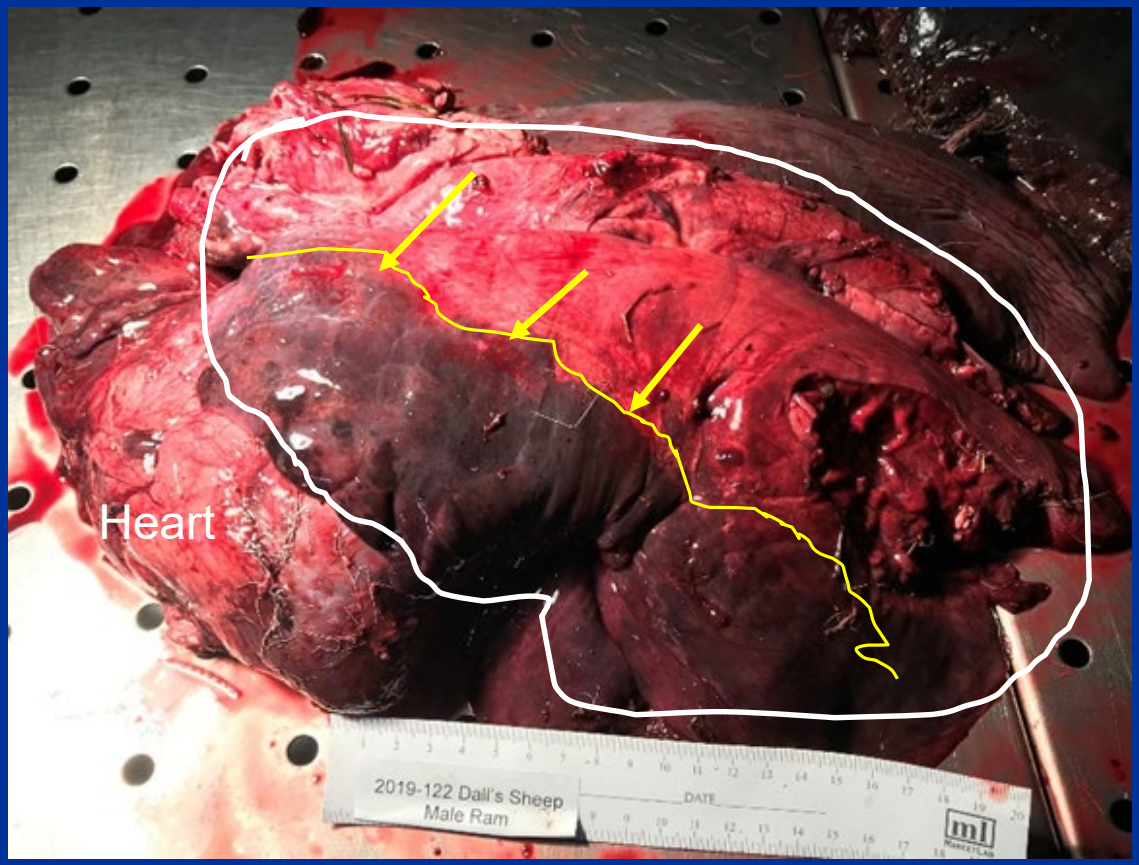
AUG 29 2004

Late July 2019- 8yo
Alaska Range East (TMA) Ram

Photo: Jeff Wells



<u>Bacteria:</u>
M. ovipneumoniae
Bibersteinia trehalosi
Trueperella pyogenes
<u>Parasites-</u>
Protostrongylus stilesi (lungworm)
Sarcocystis

Ram: Severe necrotizing bacterial bronchopneumonia

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 lymph node 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

- May 2004, 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
- Fixed paraffin block M. ovi PCR Indeterminate

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

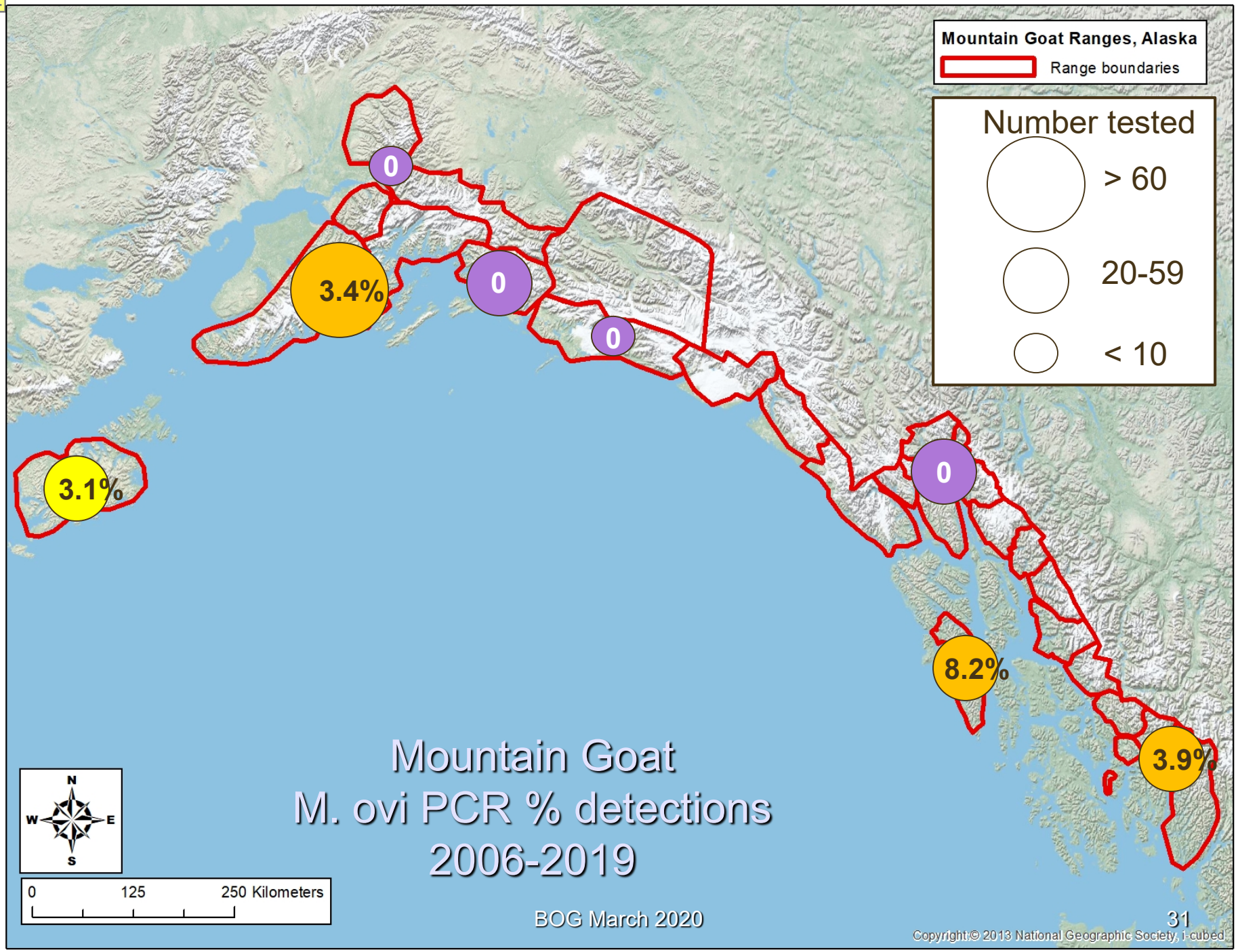
Respiratory Pathogen Surveillance – Muskox

- Lungs (2006-2017) n=31 (Eastern North Slope & Northwest)
  - All negative PCR
    - M. ovi
    - Bovine Coronavirus
    - Bovine Herpesvirus-1 (IBR)
    - Respiratory Syncytial virus
    - Bovine Viral Diarrhea 1 & 2 (Pestiviruses A & B)
  - 2018-2019 necropsies negative for M. ovi
  - *Erysipelothrix* serology retrospective: present since reintroduction

- Nasal swabs (2007-2019)
  - NW n=129, ENS n=29, SW n=17 all negative

FY20 Respiratory Pathogen Surveillance – Mountain Goat

- Nasal Swabs FY20: Hunt n=64, Capture n=21 all negative PCR for M. ovi
- Archived pneumonic lungs: n=5 2006-2011
  - All negative PCR
    - M. ovi
    - Bovine Coronavirus
    - Bovine Herpesvirus-1
    - Respiratory Syncytial virus


Mountain Goat Ranges, Alaska
Range boundaries
Number tested
> 60
20-59
< 10
0
3.4%
0
0
3.1%
0
8.2%
3.9%
N
W
E
S
0
125
250 Kilometers
Mountain Goat
M. ovi PCR % detections
2006-2019
BOG March 2020
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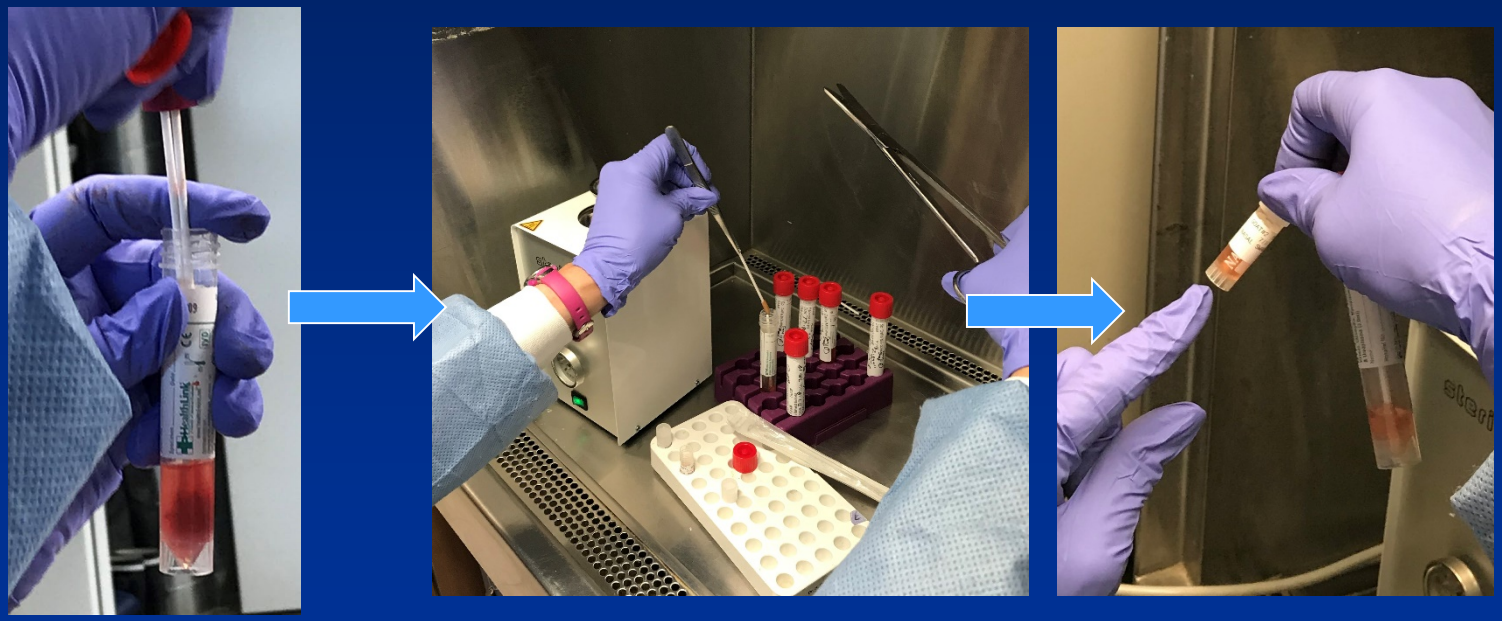
Test Concordance Study

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



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

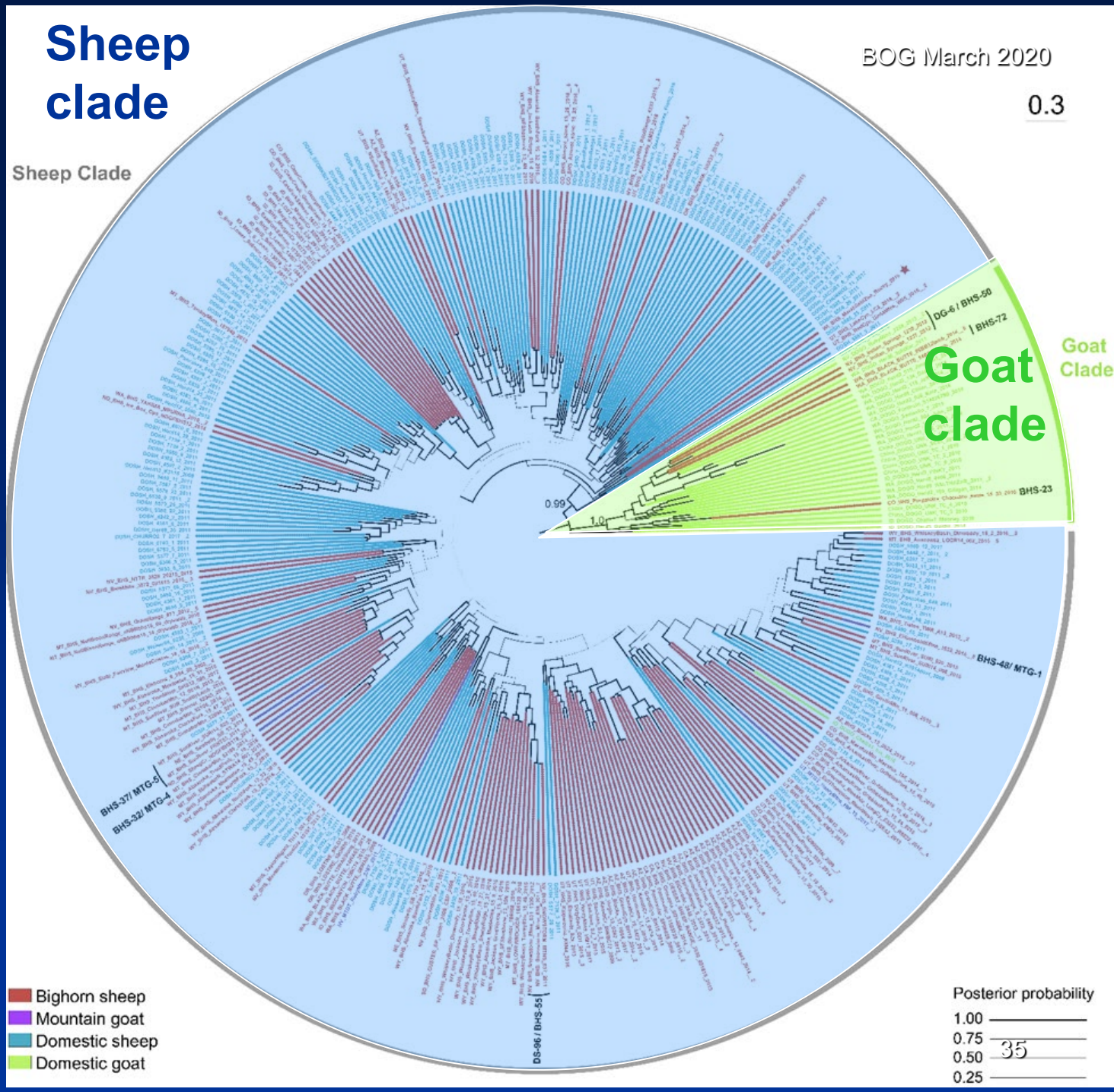
Kamath et al. 2019

M. ovipneumoniae consensus tree

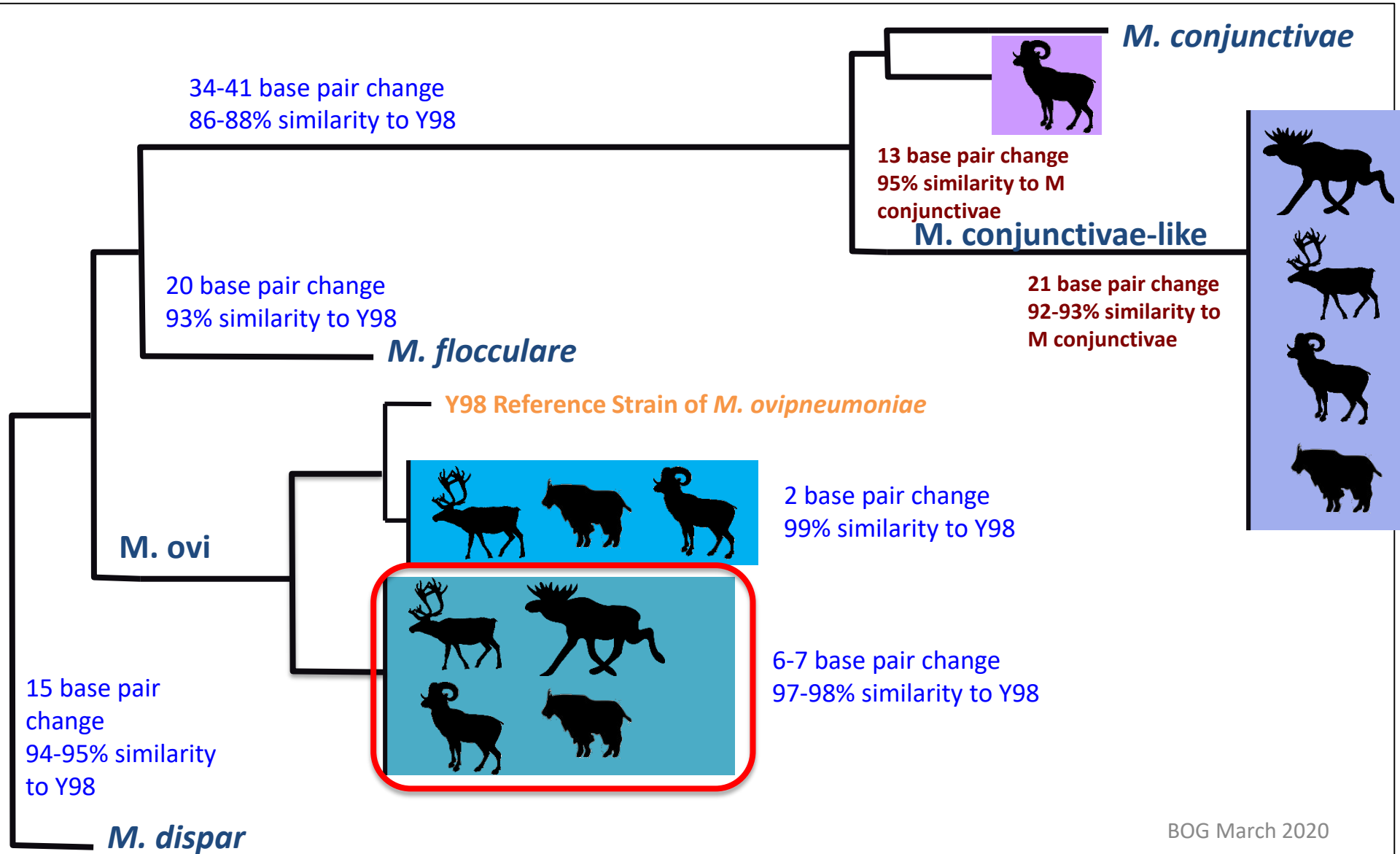
Alaskan wildlife: Have strain typed

- 11 Dall's sheep from 2 range areas sampled from 2004-2019
- 13 caribou from 4 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



Mycoplasma spp. Sequence Alignment Phylogram based on LM40



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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

Acknowledgements

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