Domestic Small Ruminants & Wild Sheep Respiratory Bacteria and Disease Research

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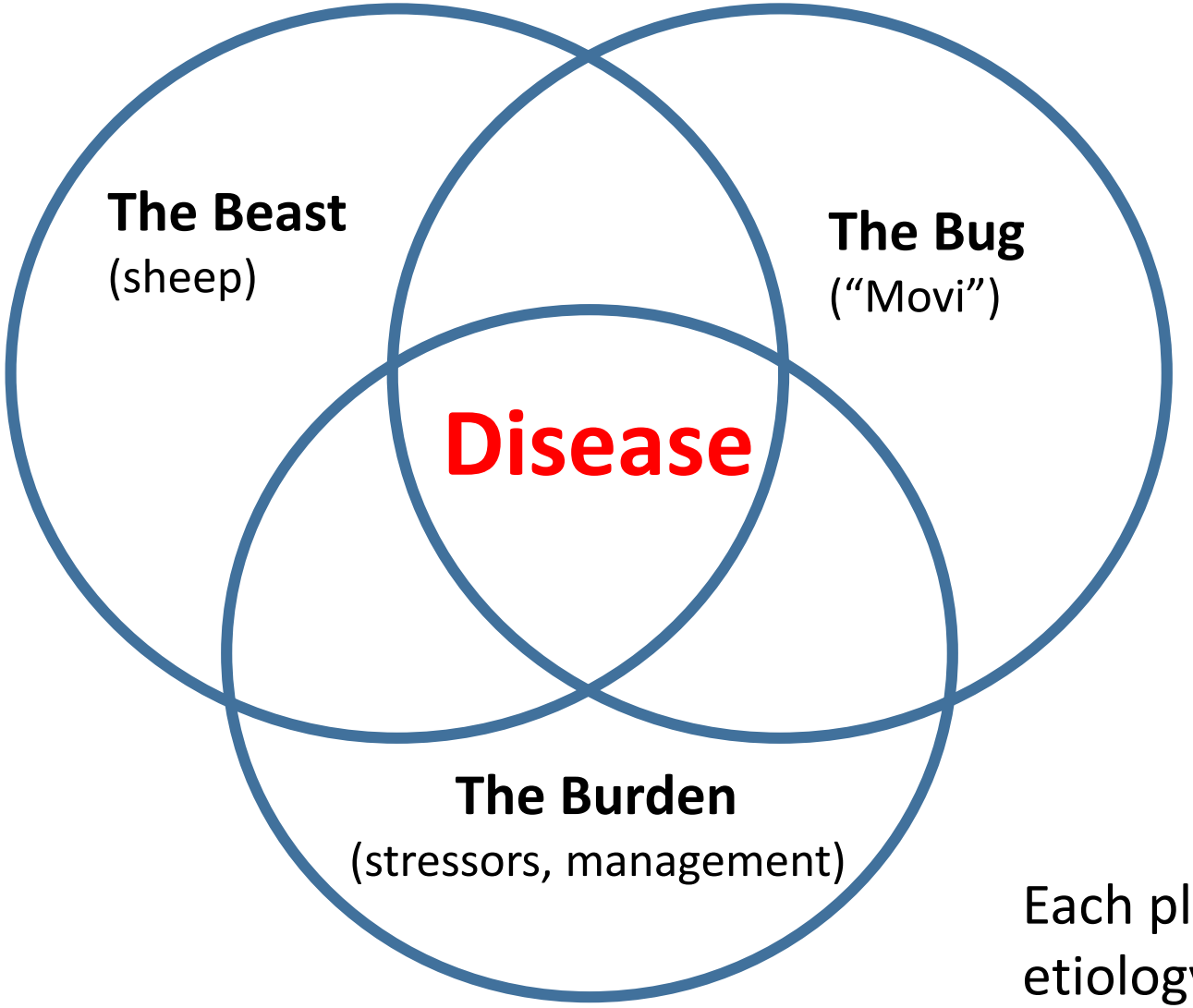
Respiratory Disease in Wild and Domestic Small Ruminants & Other Wild Ungulates

- Definitions, commonly misused terminology
- Overview of the problem and the focus on *Mycoplasma ovipneumoniae*
- Research at USDA-ARS-ADRU
- Mycoplasmas…….beyond *Mycoplasma ovipneumoniae* (“*Movi*”)

The foundation of infectious disease



Each plays a role in the etiology ("cause") of disease

Definitions

Diseases are not transmitted, pathogens are transmitted

Transmission: pathogen movement from one host to another

Disease: illness; possible outcome of transmission, dependent on

- Dose
- Host susceptibility (nutrition, stress, environment, host genetics)
- Pathogen (i.e. virulence)

Pneumonia is a disease that is caused by respiratory pathogens

Overview

Domestic–Wildlife Interface Respiratory Disease in Small Ruminants

and the focus on *Mycoplasma ovipneumoniae*

Domestic Sheep (DS) - Bighorn Sheep (BHS) Interface

Population history in the U.S./North America

Domestic sheep (DS)

60 million early 1900s (European settlement of the West)

→ 40 million in the 1940's

→ 5.25 million in 2015

Bighorn sheep (BHS)

Broad estimates of 500,000 to 2 million early 19th century

Decline → 15,000 -18,000 estimated in 1960

(hunting, habitat loss, domestic competition, **respiratory disease**)

Rebound → 72,000 estimated in 2007

85,000 estimated in 2014

(reintroductions, management efforts, controlled grazing)

Bighorn sheep (BHS) and Domestic sheep (DS)

Field reports and captive enclosure studies:

Interspecies contact $\rightarrow$ BHS respiratory disease ± fatal pneumonia (5%-95% mortality)

Captive inoculation studies:

BHS more susceptible than domestic sheep to respiratory bacterial pathogens

Proposed solution: absolute separation

Socio-economic and ecologic impacts

- DS grazing restrictions on public land allotments
  - ~48% of DS in the U.S.A. spend time on public lands
  - $800 million annual economic impact (2011 estimate)
  - 25% of the DS on Forrest Service lands have “BHS habitat overlap”
- Pressures placed on private landowners (producers & hobbyists)
- New proposals to ban use of packgoats on shared use public lands because they “may/can” carry pathogens that have been identified as agents of BHS pneumonia
- Proposal in Alaska to remove all domestic goats and sheep from the “clean list” or tall double fencing
- Herd level population-limiting pneumonia continues to impact BHS
  - Intense management efforts and removal of shared use privileges (rights?) and permits for domestic sheep and goats
  - Overall BHS populations have been increasing

DS and BHS Pneumonia

BHS

- Reports of respiratory disease date back to the 1920's
- All age outbreaks can be followed by years of disease in lambs
  - population-limiting disease
- Etiology
  - Long been debated
  - **Polymicrobial and Multifactorial**

DS

- Lambs > Adults
- Etiology
  - **Polymicrobial** (bacteria +/- viruses) or Unimicrobial
  - **Multifactorial** (colostrum, air quality, environmental stressors)

DS and BHS pneumonia-associated bacteria

***Mycoplasma ovipneumoniae* (*"Movi"*)**

Pasteurellaceae ("Pasteurellas")

- *Mannheimia haemolytica (Mh*)
  - *Pasteurella haemolytica* biotype A (prior to 1999)
- *Bibersteinia trehalosi* (*Bt*)
  - *P. haemolytica* biotype T and 3 (prior to 1990)
  - *P. trehalosi* (1990-2007)
- *Pasteurella multocida*

Anaerobic bacteria – *Fusobacterium necrophorum (Fn)*

Other aerobic bacteria (ie. *Truperella pyogenes*)

Mannheimia haemolytica

Pasteurellaceae ("Pasteurella") family member

- Easily cultured by standard laboratory methods
- Historically most commonly reported bacteria in BHS pneumonia
  (along with *Bibersteinia trehalosi*……
  remember both use to be called "Pasteurella")

Acute bronchopneumonia in compromised ruminants

- Infection with a 1° pathogen (such as *Mycoplasma ovipneumoniae*)
- Environmental stressors (air quality, crowding, shipping, other?)
- "Shipping fever" in domestic ruminants

No epidemiologic evidence to support this as the primary agent of epizootic pneumonia in wild bighorn sheep (or captive)

On the wrong track due to narrow, single bacteria, focus

Mycoplasma ovipneumoniae (“*Movi*”)

- Known for decades to be associated with domestic sheep/goat pneumonia
  - **Subacute to chronic** respiratory disease in young DS
    - Atypical pneumonia/“coughing syndrome”; otitis media
  - Associated with suboptimal environmental conditions (poor passive transfer/nutrition, environmental stressors, etc.)

- Discovered in the last decade to be highly associated with the complex phenomenon of bighorn sheep pneumonia
  - Can impact adults and lambs
  - “Pasteurellas” and other mixed bacteria found but not consistently like *M. ovipneumoniae*
  - Captive commingling studies: no *M. ovipneumoniae* → low/no mortality

Mycoplasma ovipneumoniae

- Believed to be species specific (members of subfamily Caprinae: goats/sheep/muskox)
- Fastidious organism $\rightarrow$ enrichment broth and/or PCR detection
- 1° respiratory pathogen → 2° pulmonary bacterial infection

Captive interspecies commingling studies

Species commingled	Bighorn sheep (died/total)	% death BHS	# of studies	Bacteria
DS (39)	41/43	95%	7	*Mh, Bt, Mo, A. pyogenes, Corynebacterium*
"Movi"-free DS (4)	1/4	25%	1	*Mh, Bt* (@day 90)
Domestic goats (17)	2/16	12.5%	4	*Mh*
Horse (3)	1/6	17%	1	*Pm, Strep zoo*
Cattle	1/9	11%	2	*Mh*

(Foreyt: 1982, 1989, 1990, 1994, 1996, 1998, 2009; Onderka1988; Besser2012, 2016)

Death in BHS between 8 days and 3 months following start of commingling

Confounding the matter….

DS and BHS pneumonic agents as “commensals”

M. ovipneumoniae

Upper/lower respiratory tract of subfamily *Caprinae* (sheep and goats)

- Healthy DS herds: 87% positive (453 tested)
(National Animal Health Monitoring System-Sheep2011)

- Healthy BHS herds: 4 of 32 positive (more exist, these are just those published)
- Pneumonic BHS herds: healthy carriers present (disease w/in last 10 yrs)
(Besser, Cassirer, Highland, et al. *Prev. Vet. Med*. 2012)

“Pasteurella” (including pathogenic forms)

- Upper respiratory/oropharynx in both DS and BHS

- Multiple publications support this statement

What do we know about bighorn sheep pneumonia?

Polymicrobial
(more than 1 bacteria involved)
[However, *M. ovipneumoniae* currently has strongest epidemiological evidence as being a necessary and primary agent in wild sheep pneumonia]

Multifactorial
(the presence of the bacteria in BHS alone
does NOT = disease/death)

Incompletely understood disease phenomenon

We know much less about wild thinhorn sheep

Research at ADRU-ARS-USDA (current and proposed)

- Identification of host factors in DS and BHS associated with respiratory disease and shedding of respiratory pathogens
  - *Mycoplasma ovipneumoniae* shedding (genetics)
  - Do co-infections play a role in respiratory disease? (microbiome/microbiota)
- Comparative immune system analyses to understand the difference in susceptibility to pneumonia between and amongst DS and BHS
- Impact of stress/environmental components on BHS pneumonia (known in domestics)

- ***Mycoplasma ovipneumoniae* prevalence and discovery of uncharacterized mycoplasmas**

  - Identifying hosts (are sheep and goats really the only carriers *M. ovipneumoniae*?)
    - Elk, Deer, Antelope, Moose, Caribou
  - Alaska domestic sheep and goats and wild ungulates: prevalence/distribution and genetic characterization of identified mycoplasmas
  - Pack goats: prevalence of *M. ovipneumoniae* in lower 48 states

Pack goat study – *Mycoplasma ovipneumoniae* prevalence

(Goats sampled 3 times at minimum 4 week intervals)

Mycoplasma ovipneumoniae Nasal Swab Results

USDA-ARS-ADRU Results - repeat sampling

# Tested	Detected
576 goats	38 (6.6%)
83 premises	5 (6.0%)

Detected once in 1 animal on 4 other premises

(6.25% to 57.1% prevalence on premises with *M. ovipneumoniae* detected on repeat sampling)

WADDL qPCR - duplicate nasal swab from one of the 3 collections

# Tested	Detected	Indeterminate
468 goats	18 (3.8%)	20 (4.3%)
83 premises	5 (6.0%)	9 (10.8%)

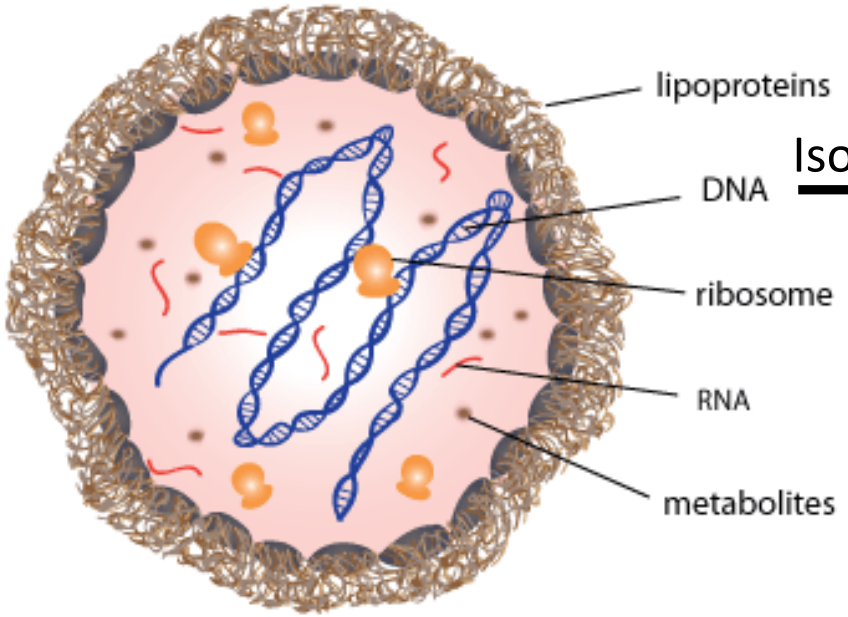
(Premises with "Detected" overlap with the "Indeterminate"; 2 of 5 premises different than ADRU results)

Pack goat study – *Mycoplasma ovipneumoniae* prevalence

- 90% of the *M. ovipneumoniae* goats were ≤1 year of age
- 77% were <6 months of age
- Additional samples from volunteer participants attending the 2016 North American Packgoat Association's Rendezvous in Oregon:
  - 27 adults and 2 kids – no detected *M. ovipneumoniae* on nasal swab samples
- Future interest in understanding the higher prevalence of shedding in kids as compared to adults

Brief overview of how nasal swabs are analyzed for mycoplasma bacteria

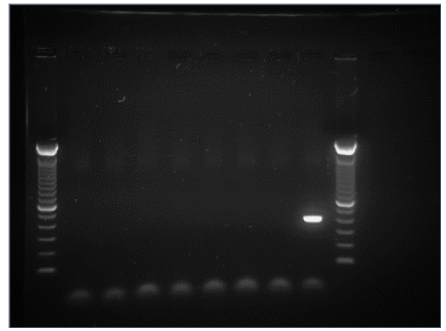
Mycoplasma



Isolate DNA

Polymerase chain reaction
(amplifies DNA)

Look at DNA



Look really close at the DNA (sequence)

TTCAATAATTTTGAATAATTTGTTCTTTTAATTCATTTT
CTCCTTGTTTAGTTTTTGTAAATTTTATTTTTTTTGTAA
ATTTTTATTTTAATGAAATTTTTAATATAATCTCAATTT
GAAATTGATTATGTACCTAGTTTTGAGAGCTCTATGTC
TGTAAAAAGACAATTAGCTCTTTCAAAACTGAACAGT

Quantitative PCR

- good for rapid detection and quantifying DNA
- cannot ‘look’ at the DNA though
- False positives and the discovery of ‘new’ mycoplasmas

Discovering uncharacterized mycoplasmas

- 2 published assays for detecting *M. ovipneumoniae* are NOT specific (commonly used prior to winter 2015)
  - qPCR Assay (Ziegler, Lahmers, Barrington, Parish, Kilzer, Baker, Besser. 2014. *PlosONE*. 9(4), e95698.)
  - Standard PCR assay (McAuliffe, Hatchell, Ayling, King, Nicholas. 2003. *Vet. Rec*. 153: 687-688.

- Led to discovering 2 as-of-yet uncharacterized *mycoplasmas* to date
  - *"Mycoplasma conjunctivae-like"* bacteria ('Mc-l')
    - Identified in domestic goats and sheep, elk, moose, bighorn sheep, white tail deer)

  - "ovipdispar"

Importance of Alaska research collaboration

- Determine the prevalence and distribution of *M. ovipneumoniae* in domestic and wild sheep and goats
  - One necessary component in assessing perceived risk to wild thinhorn sheep
- Are sheep/goats/muskox the only species that can carry *M. ovipneumoniae*?
  - Examining samples from all wild ungulates
  - Camelids have been suggested and willing to test this family of animals too
- Identify and characterize ‘new’ mycoplasma species in domestic small ruminants and wild ungulates
  - Are they pathogens?
  - Prevent false positive results when testing for *M. ovipneumoniae*

Contact information

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