



Domestic small ruminants and bighorn sheep



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What do we know about bighorn sheep pneumonia?

Polymicrobial
(more than 1 bacteria,
although 1 primary bacteria suspected)

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

Incompletely understood disease phenomenon

Bighorn sheep (BHS) and Domestic Sheep (DS)

- ❖ BHS population decline: ~1.5 million → ~72,000 over 150 years (hunting, habitat loss, domestic competition, diseases)

PNEUMONIA

- ❖ Captive contact/commingling studies and field reports:
Interspecies contact → BHS pneumonia
(currently no published reports of goats and BHS captive commingling resulting in high BHS mortality)
- ❖ Captive inoculations and *in vitro* neutrophil experiments:
BHS more susceptible than DS to pulmonary bacterial pathogens

Significance: 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
 - 25% of the DS on Forrest Service lands have “BHS habitat overlap”

- ❖ BHS population-limiting pneumonia →
continues to impede species recovery
 - Intense management efforts
 - Decades of research

DS and BHS Pneumonia

❖ DS

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

❖ BHS (wild)

- Reports of respiratory disease date back to the 1920's
- All age outbreaks often followed by years of disease in lambs
→ population-limiting disease
- Etiology
 - Long been debated
 - Evidence for **polymicrobial** and **multifactorial**

Polymicrobial

DS and BHS pneumonia-associated bacteria

- ❖ *Mycoplasma ovipneumoniae* (Mo, “M ovi”)
- ❖ Pasteurellaceae
 - *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

Mycoplasma ovipneumoniae (Mo)

- ❖ Mycoplasmataceae family member
 - Smallest of all free-living organisms, no cell wall
 - **fastidious** organisms → enrichment broth and/or PCR
- ❖ Species (subfamily) specific
 - **Reported** to cause disease only in and/or be carried by Caprinae
 - Caprinae = sheep and goats
- ❖ 1° respiratory pathogen → 2° pulmonary bacterial infections
 - Colonizes respiratory epithelium → impede mucociliary clearance
(mucociliary clearance is an escalator of sorts haul out foreign objects (ie. bacteria) that are inhaled)
- ❖ **Chronic** pneumonia in young DS (also goats)
 - Atypical pneumonia, “coughing syndrome”

Mo in BHS Pneumonia

- ❖ Historic infrequent/sporadic detection (fastidious nature)
 - *Mycoplasma* spp. in 1970; *Mo* in 1980 and 1996
(Woolf, et al. 1970; Bunch, et al. 1980; Rudolph, et al. 2007)

- ❖ High association with pneumonia in wild BHS
 - Besser, Cassirer, **Highland** et al. *Prev. Vet. Med.* 2013 (WSU)
 - Besser, **Highland**, et al. *Emerg. Infect. Dis.* 2012 (WSU)
 - Canadian Cooperative Wildlife Health Centre , 2011-12 report (BC, Canada)
 - Wolfe, et al. *J. Wildl. Dis.* 2010 (Colorado)

- ❖ **Subacute to chronic** pneumonia, otitis media
 - Time course likely dependent on host factors and 2° bacteria

- ❖ Captive commingling studies: no disease “outbreak” in the absence of *Mo*

- ❖ Healthy negative BHS herds have been identified that develop disease upon introduction of *Mo* into the herd

Mannheimia haemolytica

- ❖ Pasteurellaceae family member
 - Gram-negative coccobacillus, cultured: conventional methods (easy to culture)
- ❖ **Acute** bronchopneumonia in compromised hosts (ruminants)
 - Infection with a 1° pathogen (*Mycoplasma*, virus)
 - Environmental stressors (air quality, crowding, shipping, other?)
- ❖ Leukotoxin A (LktA)
 - Secreted leukocytotoxin (ruminant specific)
 - Primary virulence factor secreted by certain strains of *Mh* and *Bt*
- ❖ No conclusive evidence that this bacteria is the primary cause of BHS pneumonia outbreaks

Lkt+ *Mh* & *Bt* in BHS pneumonia

- ❖ Historically the most researched bacteria
- ❖ Historically two of the most commonly reported bacteria in BHS pneumonia
 - Often cultured together and/or along with other bacteria
 - Remember....easy to culture by conventional methods
- ❖ BHS pneumonia predominantly subacute to chronic
 - Pasteurellosis more typically an acute bronchopneumonia
 - May act as lethal 2° pathogen in subacute/chronic pneumonia

Fusobacterium necrophorum

- ❖ Gram-negative, rod-shaped, culture: anaerobic conditions
- ❖ Disease in ruminants: hepatic abscesses, foot rot, necrotic laryngitis
 - Secondary infections due to epithelial compromise
- ❖ Leukotoxin (Lkt)
 - Secreted leukocytotoxin
- ❖ Recently reported in association with polymicrobial BHS pneumonia
 - Non-culture methods – 16s sequencing and cloning
(Besser, **Highland**, et al. *Emerg. Infect. Dis.* 2012)

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)
(APHIS, National Animal Health Monitoring System-Sheep2011, K. Marshall, T. Besser)
- Healthy BHS herds: 4 of 32 positive
Pneumonic BHS herds: healthy carriers present (disease w/in last 10 yrs)
(Besser, Cassirer, **Highland** et al. *Prev. Vet. Med.* 2013 (WSU))

❖ Pasteurellaceae

- Upper respiratory/oropharynx
 - BHS: *B. trehalosi* > *M. haemolytica*
 - DS: *M. haemolytica* > *B. trehalosi*
- } LktA+ isolates in both species
(Tomassini, et al. *J. Wildl. Dis.* 2009 (WSU))
(Dassanayake, et al. *Vet. Microbiol.* 2010 (WSU))

❖ *Fusobacterium necrophorum*

- Normal inhabitant of mouth and gastrointestinal and urogenital tracts; in soil

Past Research

How did we get here?

Captive interspecies commingling studies

| Species commingled | Bighorn sheep (died/total) | % death | # of studies | Bacteria |
|------------------------|----------------------------|---------|--------------|-------------------------------------------------|
| DS (39) | 41/43 | 95% | 7 | <i>Mh, Bt, Mo, A. pyogenes, Corynebacterium</i> |
| <i>Mo</i> -free DS (4) | 1/4 | 25% | 1 | <i>Mh, Bt</i> (@day 90) |
| Goat (7) | 2/10 | 20% | 2 | <i>Mh</i> |
| Horse (3) | 1/6 | 17% | 1 | <i>Pm, Strep zoo</i> |
| Cattle | 1/9 | 11% | 2 | <i>Mh</i> |

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

Mh = *Mannheimia haemolytica*

Bt = *Bibersteinia trehalosi*

Pm = *Pasteurella multocida*

BHS – DS Comparative Experiments

Neutrophils (a type of white blood cell) collected from BHS & DS

- BHS neutrophils 4 – 8 x more sensitive than DS to toxin of “Pasteurellas”
(Silflow, et al. *J Wildl Dis.* 1989; 1994)

Inoculation studies (intranasal and intratracheal)

- Multiple studies reported death in BHS and no disease in DS
 - **no consideration of the immune status of the animals prior to study or BHS had “low” titers** (Dassanayake, et al. *Vet. Microbiol.* 2009; 2013)
- BHS have “defective pulmonary clearance” of *M. haemolytica*
 - **BHS had significantly lower lung and blood antibody titers to bacteria**
(Subramanian, et al. *Vet Microbiol.* 2011)

Passive transfer study indicating low transfer of antibodies - Looked specifically at antibodies for one bacteria (*M. haemolytica*)

- also reported that the bighorn ewe had low/no *M. haemolytica* titers
(Herndon, *Clin Vaccine Immunol*, 2011)

Leading to a belief (misconception?) that bighorn sheep are “immunocompromised”
Unable to handle even natural infection with leukotoxin positive “Pasteurellas”

**Current research at ADRU-ARS-USDA
Pullman, WA**

If domestic and bighorn sheep are raised equally, are their immune systems equally protective?

Domestic sheep and bighorn sheep were taken at birth

- No contact with the ewe or any other sheep
- Hand-raised (collected colostrum from ewe then milk replacer after)
- Separated by species
- Remain free of the bacteria associated with pneumonia

What we are investigating?

- Passive transfer of antibodies from ewe to lamb
- Repeat toxicity assays on neutrophils from SPF animals
- Neutrophil and macrophage function (in vitro assays)
- Examining white blood cells from each species to understand species differences
- Intranasal immunization (atomization)

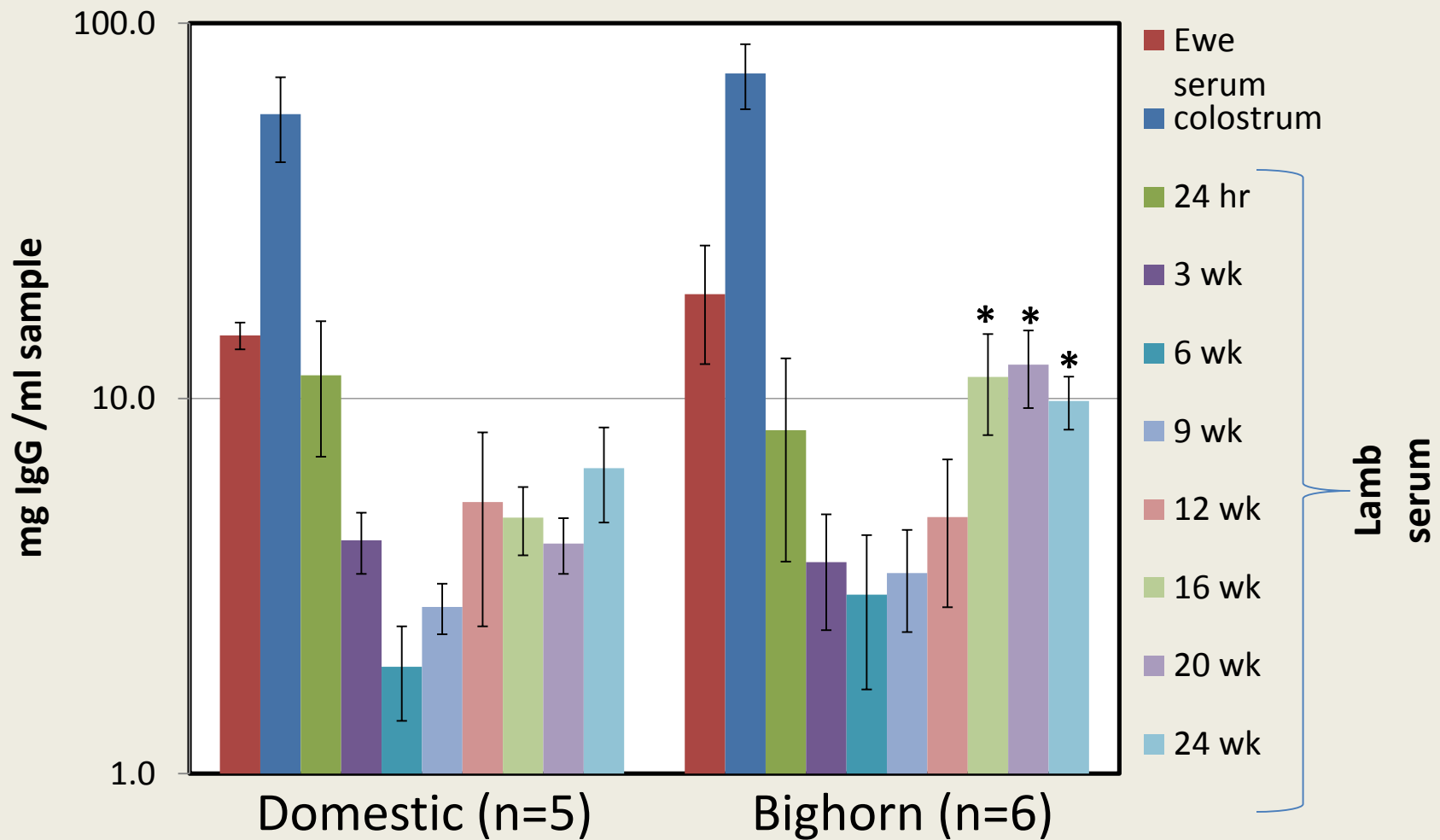
heat killed *Mycoplasma ovipneumoniae* and *Mannheimia haemolytica*

(Bacteria from lung tissue of BHS that died from pneumonia)

USDA-ARS-ADRU Research Results Summary

- Neutrophils isolated from BHS and DS raised under similar conditions are more susceptible to leukotoxin from *M. haemolytica* (similar to what was previously reported by others)
- However, BHS neutrophils are also more susceptible to the leukotoxin that is produced by the anaerobic bacteria, *Fusobacterium necrophorum*, indicating that the increased sensitivity is not bacteria specific, but rather that BHS neutrophils likely respond more rapidly/vigorously against bacteria (which can be bad)
- BHS neutrophils are at least as efficient (trend toward being better even) than are DS neutrophils in killing *Mannheimia haemolytica* bacteria when tested in a test tube, if similar immune conditions are provided
- Identified some interesting differences between BHS and DS neutrophils that may at least in part explain the different response (at least in the test tube) to bacteria exposure

Passive transfer Total IgG



* P < 0.05 (two-tailed student's t-test)

Future research planned/underway

- Comparative *in vitro* neutrophil and macrophage responses to *Mycoplasma ovipneumoniae* (ie. phagocytosis/stasis)
- Intranasal atomization and humoral immune responses to heat killed *M. ovipneumoniae* and *M. haemolytica* (Bacteria from lung tissue of BHS that died from pneumonia)
- Investigate the potential stress component of BHS disease
- Other non-Caprinae carriers of *Mycoplasma ovipneumoniae*?
 - other ungulates (deer, elk, antelope)
 - Flies
 - Rabbits

Data Base for Record and Risk Assessment

Land use

- GIS documentation or mapping of BHS herd locations and herd size
- Mapping of all public DS rangelands
- Survey all private lands within and surrounding known BHS herd ranges
 - ✓ Map locations of private lands that have DS and goats

Commensal bacteria screening in DS and BHS

- *Mycoplasma ovipneumoniae* strain typing
- Pasteurellaceae (*Mh*, *Bt*, *Pm*)

Disease documentation in DS and BHS

- Dates
- Number affected
- Symptoms
- Pathogens identified and by whom

Human interactions with BHS

- Wildlife agencies
- Other gov't and private activities
- Hunting
 - ✓ Permits issued
 - ✓ Herd size
 - ✓ # Harvested

Environment

- Weather
- Feeding stations
- Natural disasters (ie. fire)
- Non-human predators

Table 1. Bighorn sheep pneumonia die-offs, winter 2009-2010, in 5 states (MT, NV, WA, UT, WY), as of 6/21/2010.

| BHS Die-off Location | Pre die-off Estimated BHS Population Size | # BHS Culled | # Known Add'l Mortalities | Estimated % BHS Mortality | Estimated # BHS Mortalities | Known, Likely or Possible Association with Domestic Sheep or Goats, Prior to BHS Die-off |
|-----------------------------|-------------------------------------------------|--------------------|---------------------------------|---------------------------------|-----------------------------------|---------------------------------------------------------------------------------------------------|
| East Fork Bitterroot, MT | 200-220 | 80 | N/A | 50% | ~100 | Known |
| Bonner/W Riverside, MT | 160-180 | 99 | 4 | 68% | ~110 | Known |
| Lower Rock Creek, MT | 200 | 18 | N/A | 43% | 87 | Possible |
| Upper Rock Creek, MT | ~340 | 39 | N/A | 60% | ~200 | Possible |
| East Humboldt Range, NV | 160-180 | 1 | 113 | 80% | 140 | Likely |
| Ruby Mountains, NV | 160 | 1 | 36 | 65% | 100 | Possible |
| Yakima River Canyon, WA | 280 | 69 | 42 | 33% | 99 | Possible |
| N slope Uinta Mountains, UT | 50-70 | 51 | 0 | 95% | 50 | Unknown |
| Gros Ventre River, WY | 50-60 | 2 | 0 | 5% | 2 | Unknown |
| Totals | 1600-1680 | 360 | 195 | | 888 | |

(WAFWA June 22, 2010 report)



Database would help to minimize this ambiguity

and

Identify other possible disease associated factors

USDA Centralized Database Proposal

How do we collect data/information from each state?

Forrest Service

BLM

State wildlife agencies

State veterinarians

ASI members from each state

Observations from individuals

Compiled information made available for all involved

Conclusion

- Polymicrobial disease – focus for years was narrowed to *M. haemolytica* (“Pasteurella”), with no strong evidence to support this as the primary cause for epizootic pneumonia
- *Mycoplasma ovipneumoniae* – remember this bacteria
- USDA research: Alternative approach to comparatively evaluate the immune systems of BHS and DS to advance the basic understanding of immune responses to pulmonary disease agents
- Database: attempt to sort out the multifactorial component
- Are captive experiments similar to the wild disease phenomenon?

Questions:

1. Can goats be tested for bacteria associated with bighorn sheep pneumonia
Yes – nasal swabs and blood tests for antibodies
WADDL- <http://www.waddl.vetmed.wsu.edu/fee-schedule>
serology for *Mo*: \$10.00/\$15.00
nasal swab/culture (qPCR): \$40/\$60 for <4 samples; \$30/\$45 for >4 samples46630
2. Vaccine in the future? Wild and/or domestic sheep and goats?
(tough question.....nothing in the near future that I know of)
3. Can goats/sheep be silent carriers?
Yes – domestic and wild goats/sheep can be
4. Research that supports closing BHS habitat to goat packing and goat/sheep grazing?
Should goats be treated the same as sheep, as far as disease transmission? (tough questions)
5. Is there a recommended separation distance? (another tough one)

Other questions welcomed.....

- References for data/information within this presentation available upon request
- Questions, comments, feedback, suggestions, and constructive criticism welcomed

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