



**Western
Watersheds
Project**

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Working to protect and restore Western Watersheds and Wildlife

December 1, 2020

Travis Moseley, Forest Supervisor
c/o Peggy Luensmann
Lincoln National Forest Supervisor's Office
3463 Las Palomas Road
Alamogordo, NM 88310

Comments submitted electronically this date via the project website:

<https://www.fs.usda.gov/project/?project=31150>

RE: Draft Environmental Impact Statement for the Integrated Non-native Invasive Plant Management Project across the Lincoln National Forest in New Mexico

Dear Mr. Moseley and Ms. Luensmann,

These comments are submitted on behalf of Western Watersheds Project, a non-profit conservation group working to protect and conserve the public lands, wilderness, wildlife, and natural resources of the West through education, scientific study, public policy initiatives, and litigation. Our staff, board and members care deeply about the natural resources located within the Lincoln National Forest (LNF). We outline our concerns about the project below and note that we would support an alternative that does not include “controlled” or targeted livestock grazing, the use of herbicides, nor the use of mechanized or motorized equipment in Wilderness areas. We strongly encourage the Forest Service to revise the Draft Environmental Impact Statement (DEIS) to include such an alternative and to remedy the additional shortcomings we identify below.

National Environmental Policy Act (NEPA) Concerns

The DEIS lacks the necessary site-specific detail to comply with NEPA. Despite its great length, the DEIS does not sufficiently disclose the locations of proposed Non-native Invasive Plant (NNIP) treatments, baseline conditions are not adequately disclosed, impacts to species are ignored, and the DEIS fails to disclose meaningful cumulative impacts of proposed NNIP treatments on wildlife and watersheds.

NEPA’s review obligations are more stringent and detailed at the project level, or “implementation stage,” given the nature of “individual site specific projects.”¹ “[G]eneral statements about possible effects and some risk do not constitute a hard look, absent a justification regarding why more definitive information could not be provided.”²

Analyzing and disclosing site-specific impacts is critical because where, when, and how activities occur on a landscape strongly determines that nature of the impact. As the Tenth Circuit Court of Appeals has explained, the actual “location of development greatly influences the likelihood and extent of habitat preservation. Disturbances on the same total surface area may produce wildly different impacts on plants and wildlife depending on the amount of contiguous habitat between them.”³ The Court used the example of “building a dirt road along the edge of an ecosystem” versus “building a four-lane highway straight down the middle” to explain how those activities may have similar types of impacts, but the extent of those impacts – in particular on habitat disturbance – is different.⁴ Indeed, “location, not merely total surface disturbance, affects habitat fragmentation,”⁵ and therefore location data is critical to the site-specific analysis NEPA requires.

NEPA further mandates that the agency provide the public “the underlying environmental data’ from which the Forest Service develop[ed] its opinions and arrive[d] at its decisions.”⁶ “The agency must explain the conclusions it has drawn from its chosen methodology, and the reasons it considered the underlying evidence to be reliable.”⁷ In the end, “vague and conclusory statements, without any supporting data, do not constitute a ‘hard look’ at the environmental consequences of the action as required by NEPA.”⁸

CEQ’s regulations establish specific ways agencies must analyze proposed actions, including project-level decisions and including a detailed discussion of direct, indirect, and cumulative

¹ *Ecology Ctr., Inc. v. United States Forest Serv.*, 192 F.3d 922, 923 n.2 (9th Cir. 1999); see also *Friends of Yosemite Valley v. Norton*, 348 F.3d 789, 800-01 (9th Cir. 2003); *New Mexico ex rel Richardson v. Bureau of Land Management*, 565 F.3d 683, 718-19 (10th Cir. 2009) (requiring site-specific NEPA analysis when no future NEPA process would occur); *Colo. Env’tl. Coal. v. Ofc. of Legacy Mgmt.*, 819 F. Supp. 2d 1193, 1209-10 (D. Colo. 2011) (requiring site-specific NEPA analysis even when future NEPA would occur because “environmental impacts were reasonably foreseeable”).

² *Or. Natural Res. Council Fund v. Brong*, 492 F.3d 1120, 1134 (9th Cir. 2007) (citation omitted); see also *Or. Natural Res. Council Fund v. Goodman*, 505 F.3d 884, 892 (9th Cir. 2007) (holding the Forest Service’s failure to discuss the importance of maintaining a biological corridor violated NEPA, explaining that “[m]erely disclosing the existence of a biological corridor is inadequate” and that the agency must “meaningfully substantiate [its] finding”).

³ *New Mexico ex rel Richardson*, 565 F.3d at 706.

⁴ *Id.* at 707.

⁵ *Id.*

⁶ *WildEarth Guardians v. Mont. Snowmobile Ass’n*, 790 F.3d 920, 925 (9th Cir. 2015).

⁷ *N. Plains Res. Council, Inc. v. Surface Transp. Bd.*, 668 F.3d 1067, 1075 (9th Cir. 2011) (citation omitted).

⁸ *Great Basin Mine Watch v. Hanks*, 456 F.3d 955, 973 (9th Cir. 2006).

impacts and their significance; and an analysis of reasonable alternatives to the proposed action. Such analysis is required for both environmental assessments and EISs.

The Forest Service has not analyzed this project in light of the South Sacramento Restoration Project DEIS (2019), nor the Smokey Bear Habitat Restoration Project (2019). WWP and others commented on these projects and we incorporate WWP's comments here as Appendix A.

The Range of Alternatives is inadequate, as the Forest Service acknowledges at page 230 of the DEIS: "Alternative B is the only alternative that would effectively contain and control NNIP. All other alternatives would result in expanded areas of infestation of certain species and corresponding degrading effects..."

The Forest Service should consider an alternative that would reduce livestock grazing or retire livestock grazing on allotments to reduce spread of NNIPs. We also recommend the Forest Service consider an alternative that utilizes nature fire ignitions and prescribed fire, along with deferred grazing or permit retirement, as a tool to manage NNIPs. Fire in the absence of livestock results in the natural postfire recovery of native flora and fauna.⁹

WWP is deeply concerned about the proposed use of livestock ("controlled grazing" or "targeted grazing") as a biological treatment and the lack of adequate NEPA analysis. This untested, unstudied method is unsuitable for the purpose and need and indeed, is likely to contribute to the continued spread of NNIP, rather than reduce NNIP populations. We are especially concerned with the use of sheep or goats because of the impacts to bighorn sheep populations and address this issue more fully below. Here, we note simply that the impacts to bighorn sheep have not been addressed at all in the DEIS.

For this project, the Forest Service has deferred the actual analysis of impacts caused by controlled or targeted grazing until some future point in time and it is unclear if the public will ever have an opportunity to participate in that future impacts analysis process. The "analysis" provides only vague, unspecific, generalized and speculative information of impacts:

Controlled grazing as a control method has mixed impacts on native plant communities depending upon how the grazing treatment is applied, the nature of the infestation and the intermingled native plants. It is not a tool to be used where effects to non-target plants cannot be tolerated as there is nothing to keep the animal from grazing a non-target plant. Grazing can produce temporary negative effects to native plants through heavy grazing and trampling. However, studies on grazing of leafy spurge (Hanson 1994) have shown no negative effects on native species diversity after five seasons of controlled grazing. Where native woody plants are present, negative effects could be more long-term, particularly when goats are used for invasive plant control. Browsing by goats may remove several years' worth of accumulated annual twig growth. Timing, stocking rate,

⁹ Reis, S.A., Ellsworth, L.M., Kauffman, J.B., and Wroblewski, D. 2019. Long-Term Effects of Fire on Vegetation Structure and Predicted Fire Behavior in Wyoming Big Sagebrush Ecosystems. *Ecosystems* 22(2): 257–265; Wroblewski, D.W. and J.B. Kauffman 2003. Initial effects of prescribed fire on morphology, abundance, and phenology of forbs in big sagebrush communities in southeastern Oregon. *Restoration Ecology*. 11:82-90.

and duration of the grazing treatment are critical to achieve control without long-lasting negative effects to native vegetation. ***The proposed action includes the need for a site-specific project operation plan if controlled grazing is to be used, that plan would include the timing, stocking rate, and duration of the grazing treatment.***

Appropriate grazing by animals preferring NNIP species can shift the plant community toward desired plant species. Conversely, grazing can selectively reduce competitiveness of native plants, shifting the community in favor of NNIP (Vallentine and Stevens 1994, Kimball and Schiffman 2003). Most NNIP species are well adapted to invade heavily grazed areas, thereby allowing competitive advantage; and some NNIP species have chemical or physical defenses (spines) that prevent them from being utilized by livestock.

2020 DEIS at 91, emphasis added.¹⁰ And for Alternative B's impacts to woody species, the DEIS similarly fails to include actual analysis of impacts and instead discusses impacts generally:

Grazing as a tool to treat non-native invasive plants has the potential to cause temporary reduction of natural regeneration of tree species, and reduction of plant cover of woody shrubs, particularly if grazing/browsing animals are left on infestation sites after target species are depleted. Negative effects could be more long-term, particularly when goats are used. Browsing by goats may remove several years' worth of accumulated annual twig growth. Timing, stocking rate, and duration of the grazing treatment are critical to achieve control without long-lasting negative effects to native vegetation. Mature deciduous and coniferous trees would experience little or no direct or indirect effects from grazing or browsing treatments. Planted tree regeneration sites should not use grazing or browsing as a non-native invasive plant treatment. The proposed action includes the need for a site-specific project operation plan if controlled grazing is to be used, that plan would include the timing, stocking rate and duration of the grazing treatment.

2020 DEIS at 93. This generalized analysis is inadequate and to proceed to a decision on the basis of the information in the DEIS would be a violation of NEPA.

As noted in the DEIS, the goals for this project are *containment and prevention* of NNIP spread. "Containment" can be efficiently and economically accomplished through a prohibition on livestock grazing in any and all areas where NNIP are known to be located. "Prevention" can also be accomplished by eliminating livestock grazing in the LNF because prohibiting livestock from consuming NNIP and trampling areas where NNIPs are located and then moving to non-infested areas would prevent the livestock from defecating NNIP seeds and parts and would also keep seeds and plant parts stuck to hooves and fur from being transported to new locations.

As evidenced by the lack of scientific citations supporting the use of targeted livestock grazing in this DEIS and as the Forest Service must be aware, there is little literature that addresses the effectiveness of cattle for use in targeted grazing. Most of the literature regarding targeted grazing examines the impacts using sheep and goats, but even this information is lacking in this

¹⁰ Kimball, S. and P. M. Schiffman. 2003. Differing effects of cattle grazing on native and alien plants. *Conservation Biology*: 17(6): 1681-1693.

DEIS. This is major concern with the DEIS and, as we note in the section on bighorn sheep, the impacts of targeted grazing should be analyzed in detail and the decisions should be based upon a review of the best available scientific information.

To quote from Reisner *et al.* (2013): “If the goal is to conserve and restore resistance of these systems, managers should consider maintaining or restoring: (i) high bunchgrass cover and structure characterized by spatially dispersed bunchgrasses and small gaps between them; (ii) a diverse assemblage of bunchgrass species to maximize competitive interactions with *B. tectorum* (cheatgrass) in time and space; and (iii) biological soil crusts to limit *B. tectorum* establishment. Passive restoration by reducing cumulative cattle grazing may be ***one of the most effective means of achieving these three goals.***” (Emphasis added.)

While the Forest Service states that the use of livestock would be a “minor, incidental treatment method” there are no actual restrictions on when or where it could be used and the impacts have not been adequately described. USFS 2020 DEIS at 21. The literature the Forest Service cites in support of its inclusion of controlled grazing in the tool box for addressing the NNIP problem indicates that *grazing management generally* is key to preventing or delaying encroachment of NNIP (such as cheatgrass, *see* Vallentine and Stevens 1994, cited in the DEIS) and that “grazing is concluded not to be an effective general tool for cheatgrass control.” *Id.*, citing Vallentine and Stevens 1994. Furthering the argument *against* controlled or targeted livestock grazing to manage NNIP species such as cheatgrass is the fact that cheatgrass is most valuable as a spring forage (meaning the time when livestock are also most likely to eat it), which coincides with the time of year perennial cool-season grasses are most susceptible to damage by grazing. *Id.* Hoof action that accompanies livestock grazing enhances cheatgrass seed germination and emergence and the seeds are incompletely digested and thus spread by livestock droppings. *Id.* It is also critical for the Forest Service to acknowledge that grazing at a level that will control cheatgrass is also likely to significantly increase soil erosion and is harder on perennials than it is on NNIP. *Id.*

To effectively treat NNIP, livestock would be expected to preferentially graze the native bunchgrasses rather than cheatgrass, medusahead, or other NNIP.¹¹ As noted by Diamond (2009), “[t]he use of this grazing treatment should therefore be limited to degraded rangeland with little or no native perennial plant cover.”¹² The allotments where NNIP are identified are ***not*** described as having little to no native plant cover and therefore it appears that livestock should ***not*** be included as a tool to control NNIP in the LNF. Furthermore, targeted or controlled grazing would likely occur during the native perennial vegetation growing season. It is improper to use targeted or controlled grazing during the growing season of perennial native plants because of the well-known harms to bunchgrasses and other native plants. Besides the disparate

¹¹ Hempy-Mayer, K. and Pyke, D.A. 2008. Defoliation Effects on *Bromus tectorum* Seed Production: Implications for Grazing, 61 Rangeland Ecology & Management 116-123 (2008); Belsky, A.J. and Gelbard, J.L. 2000. Livestock Grazing and Weed Invasions in the Arid West Oregon Natural Desert Association: Portland, OR. April. 31 pp.

¹² Diamond, J.M., Call, C.C., and Devoe, N.. 2009. Effects of targeted cattle grazing on fire behavior of cheatgrass dominated rangeland in the northern Great Basin, USA. International Journal of Wildland Fire, 18: 944–950.

impact to native vegetation from preferential grazing, impacts to native bunchgrasses from grazing are most severe during the critical growth period for those species.¹³

Another concern is that often targeted livestock grazing is implemented to manage species such as cheatgrass well *after* cheatgrass (*Bromus tectorum*) has started to dry up and has already dropped its seeds. Livestock then trample the dried grass pushing seed into the soil, improving the seed germination of cheatgrass, creating an impact that is the opposite of the professed purpose and need for this project. On rangelands in good condition, the annual cheatgrass typically cannot outcompete the native grasses. One of the factors that protects native grasses is soil crusts. These crusts cover the soil surface in the spaces between the native bunchgrasses. They make it difficult for cheatgrass seeds to become established. However, when the soil crust is broken and disturbed by livestock across large areas of the landscape, it provides an empty niche for cheatgrass to become established. Again, the use of livestock for “managing” NNIP would appear to be contraindicated to satisfy the purpose and need for this project.

Unfortunately, where cheatgrass has gained dominance, controlled or prescribed grazing is an ineffective method of control. *Id.* Kimball and Schiffman (2003), cited by the Forest Service in the DEIS state plainly that “the strategy of livestock grazing for restoration is counterproductive. It harms native species and promotes alien plant growth.”

In the 2016 scoping notice for this project targeted grazing is proposed as a minor, incidental treatment method and that a site specific project operating plan would be developed for any treatment areas. EIS No. 20200195, Draft, USFS, NM, Integrated Non-Native Invasive Plant Management at pp. 5-6. WWP has noticed that in Arizona both the Bureau of Land Management and Forest Service both tier vegetation management and livestock grazing authorizations to programmatic EISs (PEIS) for invasive species then fail to do any site specific analysis on the impacts of those vegetation management projects or grazing reauthorizations, citing the PEIS, which doesn’t include any site specific analysis. We note here that a similar problems is likely – the current DEIS does not include sufficient site-specific analysis and promises future analyses will occur. Based on the reality and example of past projects, the Forest Service should take the time now to do the analysis necessary to fully understand the impacts of its propose NNIP management.

The Forest Service’s own DEIS argues against the use of livestock to manage NNIPs and we encourage the Forest Service to eliminate this particular tool from the management toolbox. The Forest Service must also provide additional information to ensure compliance with NEPA.

Proposed Changes to the Forest Plan are Inappropriate

¹³ Karl, M.G, “Sherm” and Chambers, J.C. 2019. Livestock Grazing Management, U.S. Forest Service, Rocky Mountain Research Station, in Crist et al., General Technical Report No. 389 (2019)), at 145; Cagney, J., Bainter, E., Budd, B., Christiansen, T., Herren, V., Holloran, M., Rashford, B., Smith, M., and Williams, J. 2010. Grazing influence, objective development, and management in Wyoming’s Greater sage-grouse habitat, with emphasis on nesting and early brood rearing. Extension Bulletin B-1203. Laramie, WY: University of Wyoming, Cooperative Extension Service; Anderson, Loren D. 1991. Bluebunch wheatgrass defoliation: effects and recovery a review. BLM, Salmon, Idaho. BLM-ID-PT-91-010-4350.

WWP strongly recommends the LNF retain the Forest Plan provision that biological controls are only applied where the biological agent can be contained within the project area. *See* USFS 2020 DEIS Table 4, page 26, modifying the Management Prescriptions Applicable to All Areas, Soil and Water (Pages 40-41) for the LNF 1986 Forest Plan. There is considerable information available on the disastrous use of tamarisk beetles in Utah and Colorado to control tamarisk (a NNIP), which resulted in the widespread invasion of tamarisk beetles well beyond the anticipated range of this introduced invasive species into Arizona. The impacts of the distribution of the tamarisk beetle are still being discovered and studied, but it is well known that the unanticipated distribution of the beetle from Utah to Arizona has had significant negative impacts on the Southwestern willow flycatcher, a species protected by the Endangered Species Act.

WWP is also concerned about the proposal to change the Forest Plan provisions for Fire and Protection (page 55), All Species (page 205 and 206), Mexican Spotted Owl (page 206A), Peregrine Falcon (page 207) because these proposed changes will have significant negative impacts to wildlife, are not a necessary aspect of the proposed action, and are unnecessarily broad in their gutting of protections currently found in the Forest Plan. USFS 2020 DEIS at pp. 27-28.

Impacts to Bighorn Sheep Were Not Considered

Decades after they were extirpated from the Sacramento Mountains and surrounding areas, bighorn sheep were restored to the LNF through a 2018 New Mexico Department and Fish and Game (NMDFG) translocation effort. Following more than five years of planning, approximately 40 bighorn sheep captured from the nearby White Sands Missile Range and the San Andreas National Wildlife Refuge were released east of Alamogordo, into the Sacramento foothills. An additional release in October of 2020 increased the population to 55-65. Bighorn sheep observations have been recorded from near the Sunspot Solar Observatory in the south to La Luz Creek in the north.

The NMDFG biologists expect the herd to grow and disperse in the near future, and have stated that future releases of bighorn sheep to bolster the population are possible. The presence of additional tracts of suitable bighorn sheep habitat in the Northern Sacramento and Guadalupe ranges indicates that bighorn sheep could be restored to portions of the Smokey Bear and Guadalupe Ranger Districts through natural dispersal or artificial translocation during the life of this project.

Domestic sheep and goats carry several pathogens that may be transmitted to bighorn sheep when the two species interact or occur in close proximity. The most significant of these pathogens is *Mycoplasma ovipneumoniae*, a bacteria that acts as a coagent in fatal pneumonia outbreaks. Other bacteria involved in pneumonia-induced die-offs include *Pasteurella multocida*, *Mannheimia haemolytica*, and *Bibersteinia trehalosi*. *Parapox* virus, which causes contagious ecthyma, and *Mycoplasma conjunctivae*, which causes keratoconjunctivitis, may also be transmitted from domestic sheep and goats to bighorn sheep.

Pneumonia in bighorn sheep has been extensively studied, including by the Forest Service, the NMDFG, and the Western Association of Fish and Wildlife Agencies (WAFWA), and there is no scientific controversy surrounding the transmissibility of fatal pathogens from domestic to wild Caprinae. Transmission of pneumonia-inducing bacteria from domestic sheep and goats to bighorn sheep has been demonstrated in laboratory and field conditions, and bacteria implicated in outbreaks where commingling was not directly observed have been traced back to domestic species known to be in the area of affected bighorn sheep.

Pneumonia is the greatest limiting factor to the recovery of bighorn sheep, and maintaining spatial and temporal separation between domestic sheep and goats and wild sheep is broadly acknowledged as the only effective way to prevent pneumonia outbreaks in bighorn herds. Pneumonia outbreaks can kill up to 90% of a bighorn sheep population, and can force wildlife managers to intervene to kill remaining members of a herd in order to prevent the further spread of the disease, leading to the local extirpation of the species. When outbreaks are less than fully fatal to the adult bighorn population, prolonged periods of poor lamb survival often occur. These periods may last years or decades, and may lead to the slow decline of the population as adults die off and are not replaced. The presence of aoudad sheep that could act as intermediate hosts for livestock pathogens increases the risk to bighorn sheep from domestic animals.

While targeted grazing by domestic sheep and goats could occur under the two action alternatives detailed in the DEIS, there is no mention of bighorn sheep or the disease risk posed to extant or potential bighorn populations by domestic sheep and goats used for targeted grazing actions anywhere in the document. The DEIS does state that a “site-specific project operation plan would be developed for the treatment area[,]” but effects to wildlife are not included in the factors that would be considered during plan development. Details of how grazing would actually occur are generally lacking throughout the DEIS, and are presumably deferred to the site-specific plans, however there is no commitment to release future project operation plans to the public for review and comment or to coordinate with NMDFG to minimize the risk to bighorn sheep during the site-specific planning process. The DEIS must be amended to address each of these issues.

The DEIS must include an analysis of the project alternatives on the nascent Sacramento Mountains bighorn sheep herd, and must consider the potential for population expansion, dispersal, and translocation to other parts of the Forest throughout the duration of the project. The DEIS must include an analysis of the potential for aoudad to act as an intermediate disease vector.

The Forest Service should consult with NMDFG regarding bighorn sheep at least annually, and should notify the agency prior to initiating any project that would include the use of sheep or goats. The Forest Service should obtain the most recent data on bighorn distribution, review the best available science, and conduct a risk assessment before releasing sheep or goats onto public lands. Specific protocols to minimize the risk to bighorn sheep from domestic sheep and goats should be developed and disclosed in the EIS.

Domestic sheep and goats should not be used for targeted grazing in areas where they pose a risk to bighorn sheep. The presence of a potential wildlife reservoir for livestock pathogens, the

exotic aoudad, increases the threat of disease outbreaks and likely renders much greater portions of the range unsuitable for domestic sheep and goat grazing.

We are providing the following references relevant to the management of bighorn sheep and the impacts associated with domestic sheep and goats. We ask the Forest Service to review these important references and incorporate this information into the analysis for this project.¹⁴

Besser, T. E., Cassirer, E. F., Highland, M. A., Wolff, P., Justice-Allen, A., Mansfield, K., Davis, M. A., and Foreyt, W. (2013). Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease. *Preventive Veterinary Medicine*, 108(2-3), 85-93. doi:10.1016/j.prevetmed.2012.11.018

Cassirer, E. F., Manlove, K. R., Plowright, R. K., & Besser, T. E. (2016). Evidence for strain-specific immunity to pneumonia in bighorn sheep. *The Journal of Wildlife Management*, 81(1), 133-143. doi:10.1002/jwmg.21172

Cassirer, F. (2018). Pneumonia in Bighorn Sheep: Risk and Resilience. *Journal of Wildlife Management*, 82(1), 32-45. doi:10.1002/jwmg.21309

Dassanayake, R. P., Shanthalingam, S., Subramaniam, R., Herndon, C. N., Bavananthasivam, J., Haldorson, G. J., Foreyt, W.J., Evermann, J. F., Herrmann-Hoesing, L. M., Knowles, D. P., and Srikumaran, S. (2013). Role of Bibersteinia trehalosi, respiratory syncytial virus, and parainfluenza-3 virus in bighorn sheep pneumonia. *Veterinary Microbiology*, 162(1), 166-172. doi:10.1016/j.vetmic.2012.08.029

Henry, A. (2019, February 27). Desert bighorn return to the Sacramento Mountains. Retrieved from <http://magazine.wildlife.state.nm.us/desert-bighorn-return-to-the-sacramento-mountains/>

Jansen, B. D. (2006). Infectious Keratoconjunctivitis in Bighorn Sheep, Silver Bell Mountains, Arizona, USA. *Journal of Wildlife Diseases*, 42(2) : 407-411, 42(2), 407-411. Observations. (2020). Retrieved from <https://www.inaturalist.org/observations?nelat=33.23008707155579>

Samuel, W. M., Chalmers, G. A., Stelfox, J. G., Loewen, A., & Thomsen, J. J. (1975). Contagious Ecthyma In Bighorn Sheep And Mountain Goat In Western Canada. *Journal of Wildlife Diseases*, 11(1), 26-31. doi:10.7589/0090-3558-11.1.26

USFS. (2015). *Bighorn Sheep Risk of Contact Tool v2 User Guide*.

WAFWA, W. (2012). *Recommendations for Domestic Sheep and Goat Management in Wild Sheep Habitat*(Rep.).

¹⁴ (all references below are attached and sent with these comments)

Miscellaneous Concerns

WWP strongly objects to the aerial application of any herbicides. Aerial application does not target specific species, is indiscriminate in where it actually lands and is very likely to damage non-target species. Drift, runoff, and overspray are extremely likely with aerial application.

The DEIS does not indicate whether or how permittees would be billed for the “extra” AUMs utilized via any targeted or controlled grazing system. Please explain how billing will occur. Even in instances where federal land managers charges fees for grazing, they do not come close to recouping expenditures by those land managers to administer the grazing program, and do not internalize the actual cost use of the public lands for grazing by private corporations, which likely approaches a billion dollars a year.¹⁵ Allowing those same private corporations to use the public lands for free simply increases the loss to the public and moves further from a market rate for livestock grazing. For example, in Idaho, implementing a targeted grazing proposal has cost taxpayers approximately \$20,000 state tax dollars to haul water to support the grazing projects. The Forest Service must disclose if and where water to support its proposed targeted grazing projects will come from, and whether permittees will pay for any costs of hauling water or if they will be borne by the public via either state or federal funding.

The maps (starting on page 8) are difficult to use. The color coding in the legend has similar shades of red/blue/yellow/green for various species of plants which makes the key less helpful. For example, is the green color found in the Smokey Bear Ranger District Dalmation toadflax or Malta starthistle? Is the Sacramento Ranger District infested with African rue, common burdock, or teasle, or all three? Charts with this information would be extremely helpful.

If prescribed burning is utilized as NNIP management tool (or if natural ignitions are allowed to burn) WWP recommends that no livestock grazing be authorized for a period of ten years in these areas to reduce the chances of continuing disturbance that can lead to cheatgrass and other NNIP invasions.

Restoration should only include the use of native grasses, forbs, and shrubs. If possible, native seed sources should be collected locally to the restoration project to preserve local genetic integrity.

WWP recommends the Forest Service continue to exclude native juniper (*Juniperus spp.*) or pinyon pine (*Pinus monophylla*) as species targeted for removal or “management” as part of this project because these native species are not invasive nor in need of treatments, except to facilitate the conversion of natural landscapes to forage for commercial livestock interests.

Please ensure that all monitoring activities taking place during adaptive management phases are made available to the public on Forest Service project websites, so that interested parties may participate in the adaptive management process and offer input.

¹⁵ Glaser, C., Romaniello, C., and Moskowitz, K. 2015. Costs and Consequences: The Real Price of Livestock Grazing on America’s Public Lands. Center for Biological Diversity (2015). 44pp. Available at https://www.biologicaldiversity.org/programs/public_lands/grazing/pdfs/CostsAndConsequences_01-2015.pdf (last visited December 1, 2020).

The adaptive management strategy should consider the permanent retirement or closure of livestock allotments within the LNF as an effective and efficient method of reducing the spread of NNIP.

WWP strongly objects to the adaptive management strategy of approving the use of new (and currently unknown and undescribed) technologies and methods for NNIP management that does not comply with NEPA requirements for public notice, review and comment. USFS 2020 DEIS at 29.

As the Forest Service is well aware, livestock and wildlife grazing can modify plant community composition and structure, and overabundant populations negatively impact rangeland–watershed function and wildlife habitats.¹⁶ Negative effects on wildlife may include avoidance of water sources by wildlife, forage loss and altered plant communities, altered bird communities, and impacts to soils and insects. *Id.* For this project, the Forest Service must fully analyze and disclose how the presence, number, and grazing intensity of livestock when used as a NNIP control will impact the native and nonnative plant communities and how the presence and authorization of livestock grazing will further the purposes of this restoration project (if at all). This is especially important for summer months when cattle tend to exhibit more intensive foraging over extensive movements and can therefore forage in place longer than native ungulates.¹⁷

The Forest Service must analyze the impacts of livestock grazing in light of the known impacts livestock grazing in xeroriparian has on riparian areas. Levick *et al.* (2008) provide a comprehensive review of the ecological and hydrological importance of such systems, which provide important habitat also for many plant species (not just riparian-dependent species), refugia for plants and animals in times of drought (and climate change), a source of water for upland wildlife, and migration/dispersal corridors.¹⁸ Further, the relationship to the riparian and xeroriparian areas to the uplands are a critical component of wildlife habitat in the project area. Upland vegetation is directly related to winter species richness and abundance of avian species.¹⁹ Overgrazing and destruction of grasslands are leading causes of bird imperilment in the

¹⁶ Danvir, Rick E. 2018. *Multiple-use Management of Western U.S. Rangelands: Wild Horses, Wildlife, and Livestock*. Human–Wildlife Interactions: Vol. 12 : Iss. 1 , Article 4. Available at: <https://digitalcommons.usu.edu/hwi/vol12/iss1/4>.

¹⁷ Clark, P.E., Johnson, D.E., Ganskopp, D.C., MartinVarva, Cook, J.G., Cook, R.C., Pierson, F.B., and Hardegree, S.P. 2017. *Contrasting Daily and Seasonal Activity and Movement of Sympatric Elk and Cattle*. Rangeland Ecology & Management Vol. 70:2, March 2017. Pp. 183-191. <https://doi.org/10.1016/j.rama.2016.09.003>.

¹⁸ Levick, L., Fonseca, J., Goodrich, D., Hernandez, M., Semmens, D., Stromberg, J., Leidy, R., Scianni, M., Guertin, D.P., Tluczek, M., and Kepner, W. 2008. The Ecological and Hydrological Significance of Ephemeral and Intermittent Streams in the Arid and Semi-arid American Southwest. U.S. Environmental Protection Agency and USDA/ARS Southwest Watershed Research Center, EPA/600/R-08/134, ARS/233046, 116 pp.

¹⁹ Strong, T.R. and Bock, C.E. 1990. Bird species distribution patterns in riparian habitats in southeastern Arizona. *The Condor* 92:866-885.

southwest.²⁰ Livestock grazing has numerous known impacts to uplands, including the effects of range developments on habitat integrity.²¹

Trespass livestock is an additional concern regarding riparian impacts and impacts to vegetation more generally. The Forest Service must adequately disclose, analyze and address trespass livestock issues and how that trespass (or unauthorized use) exacerbates habitat problems in the project area.

In the table below, we note that several of the NNIP that are considered for treatment as part of this project are either not located where targeted or controlled livestock grazing is an available tool, or targeted/controlled grazing is not an effective treatment for the species identified as a NNIP.

Adapted from the Table 1 found in the 2020 DEIS at 5.

Table 1. New Mexico state-listed noxious weeds known to occur on the Lincoln National Forest.

Common name	Scientific name	New Mexico State Class
African rue	<i>Peganum harmala</i>	B Targeted grazing ineffective (EIS at 87).
black henbane	<i>Hyoscyamus niger</i>	A Targeted grazing ineffective (EIS at 87).
bull thistle	<i>Cirsium vulgare</i>	B
Canada thistle	<i>Cirsium arvense</i>	A
cheatgrass	<i>Bromus tectorum</i>	C See Field Guide for Managing Cheatgrass in the Southwest, USDA, June 2017, TP-R3-16-04: “heavy grazing may promote infestation” (p. 3) Cheatgrass does not compete well with established perennial grasses, therefore, encouraging perennial grasses will aid in cheatgrass suppression. (p. 4) Requires grazing twice a year (spring and fall) for two consecutive years with close management of livestock to <i>reduce</i> seed production, but not eliminate it. The LNF 2020 EIS for this project states that “grazing can selectively reduce competitiveness of native plants, shifting the community in favor of NNIP (Vallentine and Stevens 1994, Kimball and Schiffman 2003). Most NNIP species are well adapted to invade heavily grazed areas, thereby allowing competitive advantage; and some NNIP

²⁰ Finch, C. Ed. 2005. Assessment of grassland ecosystem conditions in the southwestern United States: Wildlife and fish. Volume 2. USDA RMRS-GTR-135-vol.2.

²¹ Fleischner, T.L. 1994. Ecological Costs of Livestock Grazing in Western North America. Conservation Biology 8:629-644.

		species have chemical or physical defenses (spines) that prevent them from being utilized by livestock.” 2020 EIS at 91. Grazing is not an effective general tool for cheatgrass control. Vallentine and Stevens 1994.
Dalmatian toadflax	<i>Linaria dalmatica</i>	A
hoary cress	<i>Cardaria spp.</i>	A
jointed goatgrass	<i>Aegilops cylindrica</i>	C
leafy spurge	<i>Euphorbia esula</i>	A
Malta starthistle	<i>Centaurea melitensis</i>	B
musk thistle	<i>Carduus nutans</i>	C
perennial pepperweed	<i>Lepidium latifolium</i>	B
poison hemlock	<i>Conium maculatum</i>	B
Russian knapweed	<i>Acroptilon repens</i>	B
Scotch thistle	<i>Onopordum acanthium</i>	A
Siberian elm	<i>Ulmus pumila</i>	C Not treated with targeted grazing
spiny cocklebur	<i>Xanthium spinosum</i>	B Targeted grazing ineffective (EIS at 87).
spotted knapweed	<i>Centaurea maculosa</i>	A
tamarisk/saltcedar	<i>Tamarix spp.</i>	C Not treated with targeted grazing
teasel	<i>Dipsacus fullonum</i>	B
yellow starthistle	<i>Centaurea solstitialis</i>	A
yellow toadflax	<i>Linaria vulgaris</i>	A
Russian Olive		Not treated with targeted grazing

Musk thistle and teasle are the most common NNIP on livestock allotments (52 and 30 allotments, respectively and with some overlap, and out of 120 allotments total on the LNF). 2020 DEIS at 215. Because the Forest Service seems to have specific location information regarding where NNIP species are located, at least by allotment, and controlled or targeted grazing is supposed to be used only where livestock are already authorized, the analysis of impacts from targeted grazing should have included site specific analysis. Unfortunately, there are no maps of the allotments that have known NNIP infestations and there is no description of where controlled or targeted livestock grazing will occur.

The Forest Service should also consider the following information regarding the use of livestock for NNIP management:

- Reisner et al. (2013)²² stated that: “[o]ur results provide strong support for some *a priori* hypothesized mechanisms (i.e., cattle trampling reduces bunchgrass and [Biological Soil Crust] abundance) and no support for others (i.e., cattle reduce invasions by grazing *B. tectorum*).” They concluded that: “[o]ur findings raise serious concerns regarding proposals to use cattle grazing to control *B. tectorum* in these systems where remnant bunchgrass communities persist (Vallentine & Stevens 1994). In contrast, our findings support recent guidance for passively restoring resistance of these systems by reducing grazing levels (Pyke 2011).”
- Williamson et al. (2019)²³ found that grazing corresponds with increased cheatgrass occurrence and prevalence regardless of variation in climate, topography, or community composition, and provide no support for the notion that contemporary grazing regimes or grazing in conjunction with fire can suppress cheatgrass.
- Root et al. (2019)²⁴ sampled random sites and measured biocrust communities and vegetation across low, medium, and high grazing intensities. They found biocrust cover and species richness negatively related to grazing intensity, with plots with the lowest grazing intensity having the highest biocrust diversity and cover. Additionally, they found that exotic annual grasses were substantially more abundant in plots with higher grazing intensity. Their results indicated that reduction of biocrust cover and richness favored exotic annual grasses and disfavored perennial grasses, highlighting the importance of biocrust cover in maintaining site resistance to invasion by exotic annual grasses.
- Condon and Pyke (2018)²⁵ summarized that “Evidence of grazing was more pronounced on burned sites and was positively correlated with the cover of *B. tectorum*, indicating an interaction between fire and grazing that decreases site resistance.” However, even “[i]ndependent of fire, grazing impacts resulted in reduced site resistance to *B. tectorum*,

²² Reisner, M.D., Grace, J.B., Pyke, D.A. and Doescher, P.S. 2013. Conditions favouring *Bromus tectorum* dominance of endangered sagebrush steppe ecosystems. *Journal of Applied Ecology*, 50(4): 1039-1049. doi: 10.1111/1365-2664.12097

²³ Williamson, M.A., Fleishman, E., MacNally, R.C., Chambers, J.C., Bradley, B.A., Dobkin, D.S., Board, D.I., Fogarty, F.A., Horning, N., Leu, M., and Wohlfeil Zillig, M. 2019. Fire, livestock grazing, topography, and precipitation affect occurrence and prevalence of cheatgrass (*Bromus tectorum*) in the central Great Basin, USA. *Biological Invasions*. Springer Nature Switzerland.
<https://doi.org/10.1007/s10530-019-02120-8> (WWP submitted this as a supplemental submission to our scoping comments on 2/28/20 during the reopened comment period).

²⁴ Root, H., Miller, J., and Rosentreter, R. 2019. Grazing disturbance promotes exotic annual grasses by degrading soil biocrust communities. *Ecological Applications*, 0,(0), 2019, e02016.<https://esajournals.onlinelibrary.wiley.com/doi/full/10.1002/eap.2016>

²⁵ Condon, L.A. and Pyke, D.A. Fire and Grazing Influence Site Resistance to *Bromus tectorum* Through Their Effects on Shrub, Bunchgrass and Biocrust Communities in the Great Basin (USA). *Ecosystems* (2018) 21: 1416

suggesting that grazing management that enhances plant and biocrust communities will also enhance site resistance.

- Bruegger et al. (2016)²⁶ found that targeted grazing reduced fire behavior only under “moderate” fire conditions, e.g., winds <8 kmh⁻¹. Strand et al. (2014)²⁷ stated that extreme fire weather conditions, characterized by low fuel moisture and relative humidity, and high temperature and wind speed, affect wildland fires more than do fuel characteristics, and the potential role of grazing to alter fire behavior is limited.

- Livestock are a key part of the feedback loop of invasive annual grasses and fire. Courts have also recognized the clear role of livestock in the cheatgrass problem. *see W. Watersheds Proj. v. Dyer*, Nos. 4-cv-181, 2-cv-521, 2009 WL 484438, at *7, *10–11 (D. Idaho Feb. 26, 2009) (finding that livestock grazing promotes cheatgrass, which has contributed to dramatic increase in fires affecting sagebrush habitat); *W. Watersheds Proj. v. Salazar*, No. 4:08-cv- 516-BLW, 2011 WL 4526746, at *8 (D. Idaho Sept. 28, 2011) (“cheatgrass is highly flammable [and] [t]he spread of cheatgrass is exacerbated when the native perennial grass and forb community is weakened as a result of heavy livestock grazing”).

The DEIS is not sufficiently complete to allow public review and comment

We note that the DEIS section on Range Management appears to be in a more “draft” form than much of the rest of the EIS. At page 216 of the DEIS there is a heading “Resource Indicators and Measures for Alternative A” which is followed by a sub-heading “Alternative B – Proposed Action” but there is no information regarding Alternative A. We would like an opportunity to review and comment upon the completed DEIS when this section has been completed.

Additionally, the DEIS states that “[i]f controlled (targeted) grazing is used as a biological control method a site-specific project operation plan would be developed.” 2020 DEIS at 217. This is confusing because controlled or targeted livestock grazing is a part of this proposed action, it can only occur on grazing allotments where NNIPs are known to occur, so WWP and the public generally must assume that controlled or targeted livestock grazing *will in fact* occur and we do not understand why the analysis of the impacts of this NNIP management tool was not completed as part of this DEIS, nor why the specific locations (or even allotment locations) were not disclosed. Please explain.

The analysis of the impacts of each alternative for all issues (from Table 8, DEIS at 40-42) fails to adequately or accurately identify, disclose, or analyze the impacts of livestock grazing across alternatives. The analysis of Alternative C acknowledges the limitations of controlled grazing as a tool for NNIP control (“the effectiveness of...controlled grazing is more limited than chemical controls...so preventing the spread of non-native invasive plants...would be more difficult.”) but

²⁶ Bruegger, R.A., Varelas, L.A., Howery, L.D., Torell, L.A., Stephenson, M.B., Bailey, D.W. 2016. *Targeted Grazing in Southern Arizona: Using Cattle to Reduce Fine Fuel Loads*, 69.1 Rangeland Ecology & Management 43 (2016).

²⁷ Strand, E., Launchbaugh, K.L., Limb, R., Torell, L.A. 2014. Livestock Grazing Effects on Fuel Loads for Wildland Fire in Sagebrush Dominated Ecosystems. *J Rangeland Applications*. 1:35-57.

is not disclosed as a limitation or constraint for this same use in Alternative B. This oversight is repeated in the description of alternatives at pages 72-74.

Other concerns

The DEIS states that leafy spurge is controlled by targeted grazing by sheep or goats. 2020 EIS at 87. Unfortunately, the Forest Service has failed to analyze the impacts of targeted grazing by sheep or goats on bighorn sheep.

How will controlled or targeted grazing, in the uplands, impact water quality in nearby streams? Will *E. coli* levels increase? Has the Forest Service analyzed the possible impacts from increased livestock presence in watersheds with *E. coli* impairment? How will increased livestock presence impact soil runoff?

Wilderness

In 1964, Congress passed the Wilderness Act “to secure for the American people of present and future generations the benefits of an enduring resource of wilderness.” The law provided statutory protections for wilderness areas and established the National Wilderness Preservation System. The Act, among other things, mandated that wilderness areas be administered in a manner that will leave them “unimpaired for future use and enjoyment as wilderness” and that will provide for “the protection of these areas” and “the preservation of their wilderness character.”

The Wilderness Act defines “wilderness” in part: “A wilderness, in contrast with those areas where man and his works dominate the landscape, is hereby recognized as an area where the earth and its community of life are *untrammeled by man*, where man himself is a visitor who does not remain.” Wilderness is “land retaining its *primeval character and influence*, without permanent improvements or human habitation, which is protected and managed so as to *preserve its natural conditions...*”²⁸ In addition, wilderness should be “*affected primarily by the forces of nature, with the imprint of man’s work substantially unnoticeable.*”

WWP strongly opposes any alternative that allows any grubbing, masticating, tilling, mowing, or any use of power tools or mechanized equipment within designated Wilderness Areas or Wilderness Study Areas.

There are several grazing allotments located within designated Wilderness areas:

- three in the White Mountains Wilderness area (Finely, Diamond Peak, and Church Mountains), while portions of four other allotments overlap with the area (though the EIS lists only three: Dry Gulch, Lower Bonito and Loma Grande)
- portions of four grazing allotments occur in the Capitan Mountain Wilderness area (Latham, Baca, Block, Arroyo Seco, and Arabella)

²⁸ 16 U.S.C. 1131(c) (emphasis added).

The Forest Service plans to use helicopters within Wilderness areas for aerial spraying to control NNIP. This would be a violation of the Wilderness Act due to the trammeling and nonconforming and unnecessary use of motorized equipment in Wilderness. The Forest Service has misleadingly characterized the impacts of NNIP in Wilderness as more significant than helicopters and has falsely characterized crews pulling NNIPs by hand as more of an impact on recreational visitors than aerial spraying via helicopters. The impacts of helicopter use will be analyzed at a later date via a Minimum Requirements Decision Guide, Minimum Requirements Analysis or Minimum Tool Analysis. However, this provides the public with no opportunity at this time to review or comment upon the site-specific impacts and there is no assurance that the public will be notified of the development of the MRDG, MRA, or MRT.

The presence of crews using hand tools for a period of several days at a time is not more impactful to Wilderness than the noise of helicopters dropping herbicides over vast areas of the landscape to control an undisclosed and perhaps small number of NNIP that are described as on the perimeter of fire scars. The Forest Service admits the scope of trammeling under Alternative B would be greatest, but attempts to minimize the impacts because they will be a bit shorter in time.

The impacts from controlled or targeted livestock grazing are inaccurately and inadequately described in the DEIS. The impacts are described as creating a “pastoral” or “rural” scenic character. 2020 DEIS at 237. This ignores the fact that in order for controlled or targeted livestock grazing to be effective as a tool to control NNIP, the livestock must remove all or nearly all of the vegetation targeted, which creates a scenic character that is far from “pastoral” or rural, and more like a rural parking lot or dustbowl. Furthermore, targeted grazing will remove native vegetation as well as NNIP, further trammeling the Wilderness qualities and impacting visual quality, recreational experiences, and water quality.

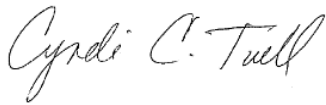
New Information

Finally, there is new information the Forest Service must incorporate into the analysis for this project. On November 24, 2020, the Environmental Protection Agency released a Draft National Level Species Biological Evaluation for Glyphosate. (available at <https://www.epa.gov/endangered-species/draft-national-level-listed-species-biological-evaluation-glyphosate#executive-summary>) In this evaluation the EPA found that glyphosate is likely to injure or kill 93% of the plants and animals protected under the Endangered Species Act and that that glyphosate adversely modifies critical habitat for 759 endangered species, or 96% of all species for which critical habitat has been designated.

Conclusion

For all of the foregoing reasons Western Watersheds Project encourages the Forest Service to revise the existing DEIS to correct the deficiencies we have identified above. We look forward to reviewing the next step in this NEPA process for this project.

Sincerely,

A handwritten signature in cursive script that reads "Cyndi C. Tuell".

Cyndi Tuell
Arizona and New Mexico Director
Western Watersheds Project

Appendix A



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Working to protect and restore Western Watersheds and Wildlife

August 16, 2019

Jodie Canfield
District Ranger
901 Mechem Drive
Ruidoso, New Mexico 88345

Sent via email this date

comments-southwestern-lincoln-smokey-bear@fs.fed.us

Smokey Bear North Restoration Project Comments

Dear Ms. Canfield,

The following comments are submitted on behalf of the staff and members of Western Watersheds Project (WWP), a west-wide nonprofit conservation advocacy organization dedicated to ending the deleterious ecological impacts of the public lands livestock grazing program. Our staff and members in New Mexico and around the west care deeply about the wildlife habitats, watershed values, ecological integrity and resilience of the lands of the area managed by the Lincoln National Forest.

WWP notes that the Notice of the Opportunity to Comment states comments are requested by Wednesday, August 16, 2019. However, this date appears to be in error because August 16, 2019 falls on a Friday. WWP believes our comments are timely submitted on this date, Friday, August 16, 2019.

This project requires the preparation of an Environmental Impact Statement (EIS)

The use of an EA for this project fails to comply with National Environmental Policy Act requirements. The geographic scope of this project covering over 130,000 acres is enough to require the preparation of an EIS. When combined with the plans to use herbicides, prescribed fire in a Wildland Urban Interface, mechanical and hand thinning treatments, the construction of temporary roads, the installation of permanent water structures for livestock, and the overlap of the project area with the Mexican gray wolf recovery area, it is clear that a Finding of No Significant Impact is not possible.

In the forthcoming analysis for this project, the Forest Service should disclose the proximity to and overlap with specially designated areas, which requires a higher level of analysis in light of the intensity and context of this specific project. Similarly, the Forest Service must disclose the presence of threatened and endangered species and designated critical habitat in the project area, which will also raise the level of analysis necessary to ensure compliance with federal regulations. *See* 40 C.F.R. §§ 1508.27(a) (context), b (intensity)).

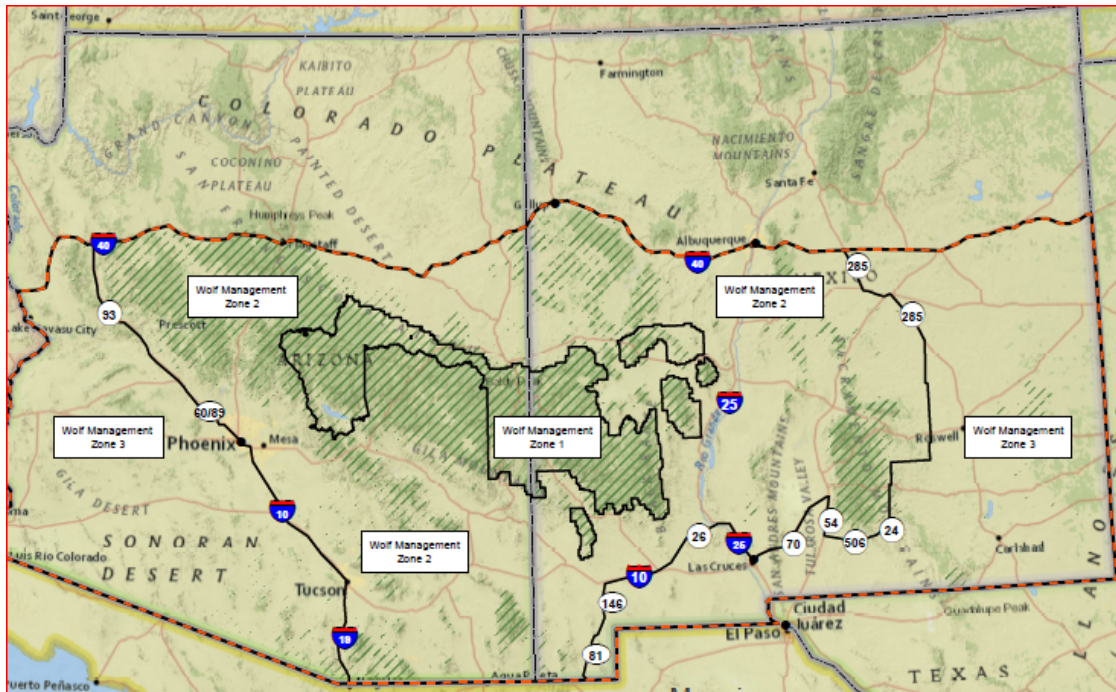
In assessing “context,” agencies must look at different geographic scales and the short- and long-term impacts of the proposed action within those different geographic scales (40 C.F.R. §1508.27(a)). In assessing “intensity,” agencies must look at the severity of the impact based on several factors:

1. The fact that impacts “may be both beneficial and adverse” and that “[a] significant effect may exist even if the Federal agency believes that on balance the effect will be beneficial.” 40 C.F.R §1508.27b(1).
2. “The degree to which the proposed action affects public health and safety.” 40 C.F.R §1508.27b (2).
3. “Unique characteristics of the geographic area such as proximity to historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas.” 40 C.F.R §1508.27b (3).
4. The degree to which the effects on the quality of the human environment are likely to be highly controversial.” 40 C.F.R §1508.27b (4).
5. The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks.” 40 C.F.R §1508.27b (5).
6. The degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about a future consideration.” 40 C.F.R §1508.27b (6).
7. Whether the action is related to other actions with individually insignificant but cumulatively significant impacts. Significance exists if it is reasonable to anticipate a cumulatively significant impact on the environment. Significance cannot be avoided by terming an action temporary or by breaking it down into small component parts.” 40 C.F.R §1508.27b (7).
8. The degree to which the action may adversely affect districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places or may cause loss or destruction of significant scientific, cultural, or historical resources.” 40 C.F.R §1508.27b (8).
9. The degree to which the action may adversely affect an endangered or threatened species or its habitat that has been determined to be critical under the Endangered Species Act of 1973.” 40 C.F.R §1508.27b (9).
10. Whether the action threatens a violation of Federal, State, or local law or requirements imposed for the protection of the environment.” 40 C.F.R §1508.27b (10).

The Forest Service must analyze the impacts of this project on Mexican gray wolves

The forthcoming analysis must acknowledge the existence and potential presence of Mexican gray wolves in and around the project area. As you can see from the maps below, this project area appears to overlap with the Mexican gray wolf Management Zones 2 and 3.

Under the currently operative 2015 Final Rule for the Revision to the Regulations for the Nonessential Experimental Population of the Mexican Wolf (80 F.R. 2512), the Mexican Wolf Experimental Population Area (MWEPA) stretches from Interstate 40 in the north to the U.S.-Mexico border in the south.



Zone 2 is an area within the MWEPA into which Mexican wolves will be allowed to naturally disperse and occupy, and where Mexican wolves may be translocated. On federal land in Zone 2, initial releases of Mexican wolves will be limited to pups less than five months old, which allows for the cross-fostering of pups from the captive population into the wild, as well as enabling translocation-eligible adults to be re-released with pups born in captivity. Some portions of the Lincoln National Forest, and possibly the project area, are located in Zone 3 of the MWEPA. Zone 3 is where neither initial releases nor translocations will occur, but Mexican wolves will be allowed to disperse and occupy.

For this project the Forest Service should disclose whether the project is located within the MWEPA Zone 2, Zone 3, or both. The possible impacts of this project on the Mexican gray wolf must be disclosed.

As part of the analysis for this project, the Forest Service must consider, analyze, and disclose the impacts of livestock grazing on Mexican wolves, especially regarding the effects on prey species. It is well understood that livestock significantly displace certain native ungulates. Wallace and Krausman, 1987. Some deer species are known to avoid cattle. Krämer 1973. Elk and deer densities can decline by as much as 92 percent in response to introduction of livestock. Clegg 1994. Because wild ungulates and cattle use the landscape in similar ways (by eating plants and moving about the landscape), but wild ungulates are more effective agents of landscape change in a reflexive relationship with ideas of land that stress natural amenities over production, (Hobson et al. 2006), the Forest Service must consider the impacts of this restoration project on the habitat preferences of ungulates. Frisina 1992.

Given that each AUM allocated to livestock effectively redirects the same forage away from native wildlife, the Forest Service should accurately discuss the public trust resources (wildlife) being replaced by private profit (livestock), and how all aspects of this restoration project will change livestock consumption as well as impacts to native ungulates.

Because the ecological costs of livestock have been clearly documented (e.g., Belsky and Blumenthal 1997, Donahue 1999, Fleischner 1994, Gillis 1991, Jones 2001, Mack and Thompson 1982, Milton et al. 1994, Painter 1995, using information garnered from reviewing published peer reviewed research and citations therein), advocates of public-lands livestock grazing (as the Forest Service appears to be for this project) must be able to demonstrate that low-impact management and ecosystem sustainability are possible, on the basis of careful use of the best available science. They must be able to demonstrate how ecological costs can be minimized. Alien taxa (including domestic livestock) and their associated infrastructure must be treated as a significant ecological stress, and negative impacts on native plants and animals, on soils and soil organisms and on all other aspects of impacted ecosystems must be anticipated and minimized. This can only be done if management decisions are made based on knowledge of the impacted flora, fauna, and ecosystems, and a management program firmly grounded in the best available science, not unsubstantiated opinions, misunderstanding, and misinformation.

As the Forest Service is well aware, livestock and wildlife grazing can modify plant community composition and structure, and overabundant populations negatively impact rangeland–watershed function and wildlife habitats. Danvir, 2018. Negative effects on wildlife may include avoidance of water sources by wildlife, forage loss and altered plant communities, altered bird communities, and impacts to soils and insects. *Id.* For this project, the Forest Service must fully analyze and disclose how the presence, number, and grazing intensity of livestock will impact the native and nonnative plant communities and how the presence and authorization of livestock grazing will further the purposes of this restoration project (if at all). This is especially important for summer months when cattle tend to exhibit more intensive foraging over extensive movements and can therefore forage in place longer than native ungulates. Clark et al. 2017.

Riparian

Livestock grazing is and has been a primary cause of stream and riparian habitat degradation in the western United States. The negative impacts of livestock grazing in riparian areas have been well documented. Poff, et al. 2011, Elmore and Kauffman, 1994. The scientific literature reveals that livestock grazing negatively affects water quality and seasonal quantity, stream channel morphology, hydrology, riparian zone soils, instream and streambank vegetation, and aquatic and riparian wildlife. Belsky et al. 1999, Ohmart 1996, Elmore and Kauffman 1994. Invertebrate and small mammal habitat is improved by livestock exclusion from riparian areas. See, e.g. Herbst 2011, Hayward et al. 1997. There is evidence of the benefits of livestock exclusion in southern Arizona with scientifically documented evidence of the improvements to riparian areas post-livestock exclusion from the San Pedro Riparian National Conservation Area (SPRNCA). From riparian canopy forest recovery to the increases in avian abundance, the scientific analyses of post-grazing effects in the SPRNCA form a strong record of the benefits of livestock exclusion that must be considered by the Forest Service while determining whether to authorize livestock grazing on these allotments where doing so will impact riparian areas. *See Appendix A, Annotated bibliography of scientific research specific to livestock exclusion in riparian areas.*

The cessation of livestock grazing in riparian areas can increase the abundance of small mammals that require dense vegetation. Soykan, et al. 2009. The substantial increase of plant cover at low height intervals that followed the removal of livestock from southwestern riparian areas can substantially increase the abundance of small mammal species that prefer cover characteristic of grassland or riparian woodland habitats. Soykan, et al. 2009, citing Duncan 1988; Krueper et al. 2003. These benefits must be disclosed and analyzed as part of this restoration project.

The Forest Service must analyze the impacts of livestock grazing in light of the known impacts livestock grazing in xeroriparian has on riparian areas. Levick et al. (2008) provide a comprehensive review of the ecological and hydrological importance of such systems, which provide important habitat also for many plant species (not just riparian-dependent species), refugia for plants and animals in times of drought (and climate change), a source of water for upland wildlife, and migration/dispersal corridors. Further, the relationship to the riparian and xeroriparian areas to the uplands are a critical component of wildlife habitat in the project area. Upland vegetation is directly related to winter species richness and abundance of avian species. Strong and Bock, 1990. Overgrazing and destruction of grasslands are leading causes of bird imperilment in the southwest. Finch, C. *Ed.* 2005. Livestock grazing has numerous known impacts to uplands, including the effects of range developments on habitat integrity. Fleischner 1994.

Trespass livestock is an additional concern regarding riparian impacts and impacts to vegetation more generally. The Forest Service must adequately disclose, analyze and address trespass livestock issues and how that trespass (or unauthorized use) exacerbates habitat problems in the project area.

Invasive species

Livestock grazing promotes the spread and colonization of alien plants, which can increase fire frequencies. Billings 1990, Billings 1994, Rosentreter 1994, Belsky and Gelbard 2000, Kimball and Schiffman 1993. Disturbance is a reliable indicator of alien dominance in vegetation composition, and livestock grazing is a significant disturbance. Brooks and Berry 2006. Further, weed invasions are strongly associated with livestock watering sites. Brooks et al 2006. The Forest Service must analyze the cause and effect relationship of livestock grazing with the woody vegetation. See, e.g. Bahre and Shelton 1993. How livestock grazing impacts invasive species of plants is critical in understanding the relationship between livestock grazing and fire in the project area.

Climate Change

The forthcoming analysis must include information about the impacts of the project on the environment *in light of the compounding impacts of climate change*. How will this project exacerbate the already alarming impacts associated with the impacts of climate change on game species, threatened and endangered species, on Management Indicator or Special Status species? How will fencing and other related infrastructure associated with this project further fragment the landscape and how will this impact species already harmed by the rapid on-the-ground changes associated with climate change?

Livestock Waters

The forthcoming analysis should identify the area of disturbance associated with existing and proposed livestock waters. The Forest Service must also analyze the direct and indirect impacts of livestock waters on species in the project area, including species listed as threatened or endangered.

As the Forest Service is aware, the impacts of livestock waters on native aquatic wildlife are significant. In some cases, stocktanks are used to stock nonnative fish for sportfishing, or they may support other nonnative aquatic species such as bullfrogs or crayfish. In cases where stocktanks are in close proximity to live streams, they may occasionally be breached or flooded, with nonnative fish escaping from the stocktank and entering stream habitats. Hedwall and Sponholtz 2005, pp. 1–2; Stone *et al.* 2007, p. 133, cited in 2012 Fed. Reg. 77, 36:10817.

For each water development, the Forest Service should disclose the volume of water to be pumped or moved, the dates pumping is allowed, and actions to be taken if the dates or volumes are exceeded. The analysis should also disclose the anticipated noise impacts from any pumping equipment, and the potential for hazardous materials spills. The public needs to know how many gallons of water will be removed from streams and springs to fill the new water developments and what impact that will have on nearby streams and springs and the associated vegetation and wildlife.

The Forest Service must disclose which allotments are included in this project and specifically, which permittees will benefit from the development of new livestock waters. The costs of the new waters should be disclosed, along with the income generated from the livestock grazing permits annually.

Rare Plants

The Forest Service should compare the known plant species in the project area to the New Mexico rare plant lists or the Forest Service sensitive species lists. The project record should include a list of plant collections found in all of the allotments that are part of this project from the University of New Mexico Rare Plant List (<http://nmrareplants.unm.edu/rarelist.php>). The Forest Service should compare these lists to see if there are any plants that require further analysis. Is there a plan to monitor for impacts to these species and if so, what actions will be taken if impacts occur?

Permit Retirement

The Forest Service must consider retiring livestock grazing permits as one tool for forest restoration, in addition to analyzing the impacts of livestock grazing as an impediment to forest restoration.

Road Decommissioning and Obliteration

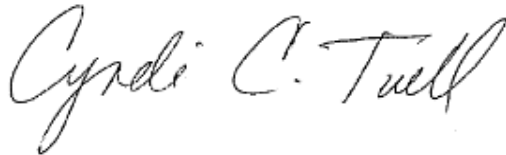
WWP supports the decommissioning and obliteration of motorized routes as part of this project as this will protect wildlife habitat, improve watershed condition, and provide for a more resilient ecosystem.

Conclusion

WWP encourages the Forest Service to acknowledge the deeply harmful impacts of livestock grazing in the Smokey Bear Ranger District and how livestock grazing is exacerbating habitat problems that this project seeks to remedy.

Thank you for your consideration of our comments. We look forward to participating in the next phase of this project.

Sincerely,



Cyndi C. Tuell
Arizona and New Mexico Director
Western Watersheds Project
520-272-2454
cyndi@westernwatersheds.org

ATTACHMENTS

Appendix A, Annotated bibliography of scientific research specific to livestock exclusion in riparian areas.

REFERENCES

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Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease

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ABSTRACT

Pneumonia of bighorn sheep (*Ovis canadensis*) is a dramatic disease of high morbidity and mortality first described more than 80 years ago. The etiology of the disease has been debated since its initial discovery, and at various times lungworms, *Mannheimia haemolytica* and other Pasteurellaceae, and *Mycoplasma ovipneumoniae* have been proposed as primary causal agents. A multi-factorial “respiratory disease complex” has also been proposed as confirmation of causation has eluded investigators. In this paper we review the evidence for each of the candidate primary agents with regard to causal criteria including strength of association, temporality, plausibility, experimental evidence, and analogy. While we find some degree of biological plausibility for all agents and strong experimental evidence for *M. haemolytica*, we demonstrate that of the alternatives considered, *M. ovipneumoniae* is the best supported by all criteria and is therefore the most parsimonious explanation for the disease. The strong but somewhat controversial experimental evidence implicating disease transmission from domestic sheep is consistent with this finding. Based on epidemiologic and microbiologic data, we propose that healthy bighorn sheep populations are naïve to *M. ovipneumoniae*, and that its introduction to susceptible bighorn sheep populations results in epizootic polymicrobial bacterial pneumonia often followed by chronic infection in recovered adults. If this hypothesized model is correct, efforts to control this disease by development or application of vectored vaccines to Pasteurellaceae are unlikely to provide significant benefits, whereas efforts to ensure segregation of healthy bighorn sheep populations from *M. ovipneumoniae*-infected reservoir hosts are crucial to prevention of new disease epizootics. It may also be possible to develop *M. ovipneumoniae* vaccines or other management strategies that could reduce the impact of this devastating disease in bighorn sheep.

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1. Introduction

Philosophers of science have long debated the process of causal inference, coming to a current consensus following the work of Karl Popper and others that proof of hypothesis is essentially unattainable, and that it is instead hypothesis falsification that drives scientific progress (Popper,

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1959). The cause of a disease has been defined as an event, condition, or characteristic that plays an essential role in producing the disease and, following Popper, the causal effect of any single factor can only be understood in relationship to conceivable alternatives (Rothman, 1986). Bighorn sheep pneumonia exemplifies the difficulty of causal inference even in the case of a dramatic infectious disease characterized by high morbidity and mortality, and the cause(s) of this disease have been subject to decades of debate and controversy. In this paper, we compare the evidence for several candidate causal microbial agents that have been proposed for epizootic pneumonia of bighorn sheep, including lungworms, Pasteurellaceae (especially *Mannheimia haemolytica*), and *Mycoplasma ovipneumoniae*, using widely accepted criteria for causality of disease, including strength of association, temporal relationships, biological plausibility, experimental evidence and analogy (Rothman, 1986).

1.1. Background

Bighorn sheep vanished from much of their historic range in North America during westward expansion in the early 20th century (Dice, 1919; Grinnell, 1928; Buechner, 1960). The precipitous decline in numbers, from 1.5–2 million in the 19th century to 15–18,000 in the United States by 1960 (Buechner, 1960) was not a unique phenomenon, as many other wildlife species' populations were similarly devastated during this era. However, the complete extirpation of bighorn sheep from much of their range, the slow rate of recovery despite intensive management efforts, and the recent listing of several U.S. populations as federally endangered (USFWS, 1998, 2000) sets them apart from most other North American ungulates.

As with other species of wildlife, market hunting and competition with livestock for forage contributed to the decline of bighorn sheep (Spencer, 1943; Buechner, 1960). However, an unusual correlation between the introduction of domestic sheep (*Ovis aries*) and the rapid disappearance of bighorn sheep was noted by early investigators (Grinnell, 1928; Schillenger, 1937; Marsh, 1938). Pneumonia was recognized as an important cause of the decline by the turn of the 20th century, and remains the most significant disease impeding recovery (Rush, 1927; Buechner, 1960; Gross et al., 2000; Cassirer and Sinclair, 2007). Pneumonia outbreaks in previously healthy bighorn sheep populations typically affect all ages of animals, result in 30–90% mortality, and are nearly always followed by at least several years of annual pneumonia outbreaks restricted to lambs that dramatically reduce population growth (Spraker et al., 1984; Ryder et al., 1992; Cassirer et al., 1996; George et al., 2008). Sporadic or continuous pneumonia events can persist in both adults and lambs in interconnected populations for many years, limiting population growth at best and potentially leading to extinction at worst (Cassirer and Sinclair, 2007).

Pathologic descriptions of bighorn sheep pneumonia were first provided in the first half of the 20th century (Rush, 1927; Marsh, 1938). Rush described a chronic pneumonia and noted lung adhesions, pus, “dark ulcers” and

“a white strip around the edge of the lobes” in sheep necropsied in an all-age pneumonia outbreak that killed about 75% of the population on a portion of the Sun River game range in Montana in 1925. Marsh described a chronic pneumonia associated with lungworms (*Protostrongylus stilesi*), *Corynebacterium* (now *Trueperella*) *pyogenes* and *Pasteurella* spp. in winter pneumonia mortalities on the Sun River game range and Glacier and Yellowstone National Parks in the 1920s and 1930s, and an acute bacterial pneumonia associated with *Pasteurella* spp. and *T. pyogenes* in a high mortality summer pneumonia event affecting 2- to 3-month-old lambs at the National Bison Range. A similar variety of pathologies and agents continues to be observed in bighorn sheep pneumonia cases. More recently, additional lesions including rhinitis, otitis media, sinusitis, tracheitis, pleuritis, broncholar hyperplasia, and bronchiectasis have been associated with this disease (Cassirer and Sinclair, 2007; Besser et al., 2008). *M. ovipneumoniae* and respiratory viruses, especially parainfluenza-3 and respiratory syncytial virus, have also been added to list of potential pathogens (Aune et al., 1998; Weiser et al., 2003; Rudolph et al., 2007; Besser et al., 2008).

While there has been a lack of clarity surrounding the pathogens associated with bighorn sheep pneumonia, experimental trials commingling bighorn and domestic sheep have had very clear results: nearly all bighorn sheep (88 of 90, 98%) commingled with domestic sheep in 11 published studies conducted between 1979 and 2009 died of pneumonia, while the domestic sheep remained healthy, as summarized in Besser et al. (2012a). In marked contrast, most (52 of 56, 93%) bighorn sheep survived similar commingling with non-ovine ungulates, including cattle, horses, deer, elk and llamas, also summarized in Besser et al. (2012a). Therefore, candidate agents enzootic in domestic sheep have been and remain a logical focus of etiologic investigations of bighorn sheep pneumonia.

The epidemiology of emerging diseases in wildlife is often unclear and causative agents are often elusive (McCallum and Dobson, 1995; Laurance et al., 1996). However, pneumonia in bighorn sheep is particularly unusual in that during nearly a century of investigation and despite the occurrence of disease outbreaks with dramatically high morbidity and mortality, no specific pathogen has been strongly or consistently associated with the disease. This rather confusing situation further led to development of the concept of a respiratory disease complex lacking a single causal agent and due instead to environmental or physiological stressors predisposing animals to invasion of the lungs with a combination of agents (Spraker et al., 1984; Rudolph et al., 2007). However, little evidence has been found for a correlative or causal relationship of physiological (Kraabel and Miller, 1997; Goldstein et al., 2005) or environmental stressors (Monello et al., 2001) and pneumonia in bighorn sheep. Thus a long history of conflicting and changing ideas about etiology and epidemiology and a lack of systematic examination of the evidence for causality have produced significant roadblocks to understanding and managing pneumonia in bighorn sheep.

2. The role of lungworms

Lungworms were one of the first agents proposed as causal agents for pneumonia in bighorn sheep. Two species of lungworm, *Protostrongylus ruscii* and *P. stilesi*, are native parasites of North American wild sheep. The worms have a multi-host life cycle that includes infection of various genera of land snails during larval stages. An early hypothesis about the etiology of bighorn sheep pneumonia was that lungworms (specifically *P. stilesi*) were primary agents that caused pneumonia directly or precipitated secondary bacterial infection in the lungs (Marsh, 1938). This disease was referred to as the bighorn sheep lungworm–pneumonia complex (Buechner, 1960; Forrester, 1971).

2.1. Strength of association

The association of lungworms with pneumonia is weak. The principal evidence in support of the lungworm hypothesis was the observation of lungworms and associated inflammation within pneumonic tissues of most affected bighorn sheep (Marsh, 1938; Forrester and Senger, 1964b; Forrester, 1971; Demartini and Davies, 1977). However, lungworm infestations are ubiquitous among wild sheep, including the northern thinhorn (Dall's) sheep (*Ovis dalli*) in which pneumonia epizootics have not been reported (Jenkins et al., 2007) as well as in most bighorn sheep populations unaffected by pneumonia (Forrester and Senger, 1964b; Hibler et al., 1982; Festa-Bianchet, 1991).

2.2. Plausibility

It is plausible that lungworms could predispose bighorn sheep to bacterial pneumonia. Lungworm larvae are frequently visible in pneumonic lung tissues of affected bighorn sheep, often adjacent to histopathologic lesions including bronchiolar hyperplasia and lymphocyte cuffing of airways commonly seen in bighorn sheep pneumonia. Furthermore, lungworm larvae are transmitted transplacentally to bighorn lambs, consistent with pre-weaning pneumonia mortality often seen in this disease (Forrester and Senger, 1964a; Hibler et al., 1974). However, bighorn sheep pneumonia lesions primarily affect anterior-ventral lung lobes while verminous pneumonia is expected to be broadly distributed with the most severe lesions usually occurring in the diaphragmatic lobes (Radostits et al., 2007). Finally, lungworms do not provide a plausible explanation for the well-documented transmission of pneumonia to bighorn sheep following contact with domestic sheep, given the different species of protostrongylids that typically affect these different host species (Panuska, 2006).

2.3. Experimental evidence

Experimental evidence is not supportive of the lungworm hypothesis. Experimental challenge of susceptible bighorn sheep with very high doses of infective lungworm larvae failed to produce any observed disease, refuting the possibility that bighorn sheep might be especially susceptible to lungworm induced pneumonia (Buechner,

1960; Forrester, 1971; Samson et al., 1987). Furthermore, anthelmintic treatments that effectively reduced lungworm burdens (Schmidt et al., 1979) were unsuccessful at improving lamb survival or preventing epizootics (Risenhoover et al., 1988; Goldstein et al., 2005).

Perhaps reflecting this evidence, by the 1980s the model of the lungworm–pneumonia complex had changed to describe a disease in which lungworm infection primarily affected lambs as a consequence of poor habitat conditions and other stressors (Spraker et al., 1984; Risenhoover et al., 1988; Easterly et al., 1992).

3. The role of Pasteurellaceae

M. haemolytica, *Bibersteinia trehalosi* (formerly designated *Pasteurella haemolytica* types A and T, respectively) and *P. multocida*, all commonly associated with pneumonia of domestic ruminants (Griffin et al., 2010), have been frequently isolated from the lungs of bighorn sheep with pneumonia and have been implicated as causal agents since the 1920s (Rush, 1927; Queen et al., 1994; Weiser et al., 2003; Rudolph et al., 2007). Among these bacteria, most research attention has been directed to leukotoxin positive strains of *M. haemolytica*, a bacterium hypothesized to be transmitted from domestic sheep to bighorn sheep and involved in the etiology of the disease, although the extent of the hypothesized involvement has ranged from “contribute to a pneumonia complex. . .” (Wild and Miller, 1991) to “consistently causes fatal bronchopneumonia in bighorn sheep” (Herndon et al., 2011). Other aerobic bacteria are also frequently isolated from pneumonic bighorn sheep lungs, including *T. pyogenes* (particularly from bighorn sheep with chronic lesions and/or pulmonary abscessation) (Queen et al., 1994) and an as yet unnamed *Moraxella* sp. (unpublished data, GenBank JQ814872), but these have generally been considered incidental findings rather than causative agents.

3.1. Strength of association

Strength of association is weak. Prevalence of specific Pasteurellaceae pathogens in lung tissues of pneumonic bighorn sheep, as detected by bacterial cultures or by polymerase chain reaction (PCR) tests, though generally high, is similar to that detected in lungs of healthy bighorn sheep that have died from causes other than pneumonia, and therefore do not reflect strong association with disease (Cassirer, 2005; Kelley et al., 2007; Besser et al., 2012b). Leukotoxin positive *M. haemolytica* can be detected, albeit at lower frequency than in domestic sheep, in the nasopharyngeal flora of healthy bighorn sheep in populations unaffected by pneumonia (Foreyt and Lagerquist, 1996; Kelley et al., 2007; Tomassini et al., 2009). Using culture and PCR, Besser et al. (2012b) documented frequent detection of *M. haemolytica* (57%), *B. trehalosi* (77%) and *P. multocida* (48%) in the lung tissues of 44 pneumonic animals from eight bighorn sheep pneumonia outbreaks in Montana, Nevada, Oregon, and Washington. Normal lung tissues from five bighorn sheep in healthy populations showed similar prevalence of the same three Pasteurellaceae (60%, 80% and 20%, respectively) using the same methodologies.

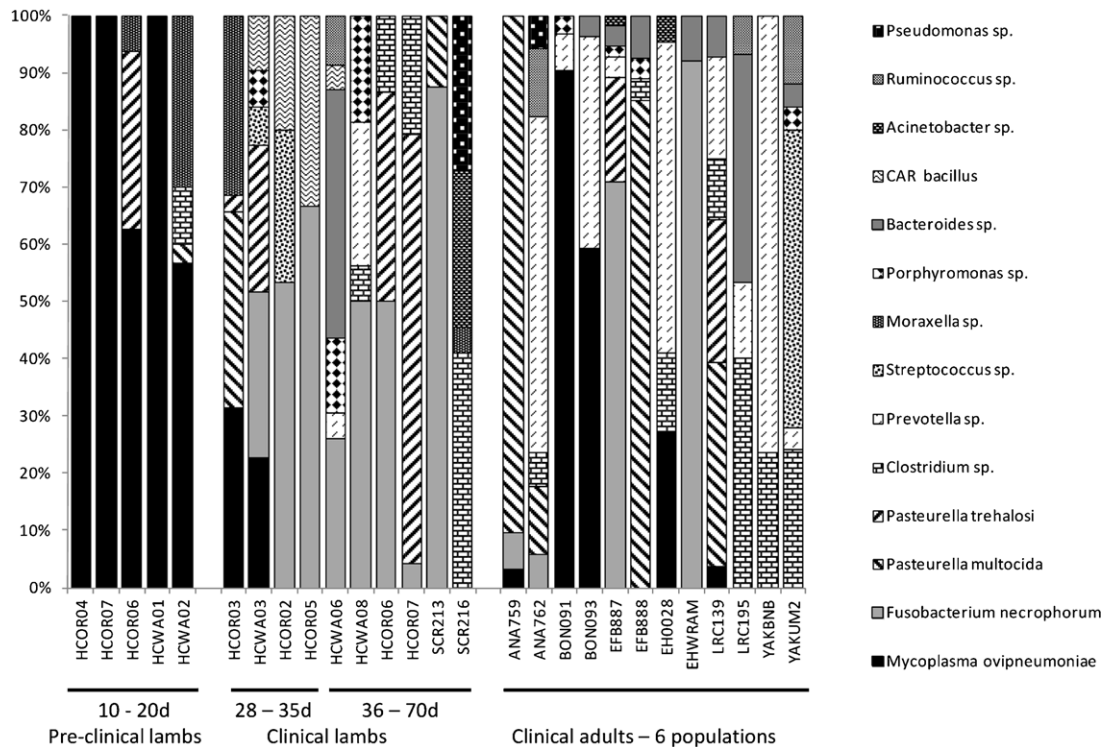


Fig. 1. Principal bacterial components of pneumonic bighorn sheep lung tissues based on 16S rDNA clone library analysis (Besser et al., 2008, 2012b). The X-axis legend specifies populations of origin of the bighorn sheep from which the tissues were sampled: HCOR = Hell's Canyon OR; HCWA = Hell's Canyon, WA; SCR = Spring Creek SD; ANA = Anaconda MT; BON = Bonner MT; EFB = East Fork Bitterroot MT; EH = East Humboldt NV; LRC = Lower Rock Creek MT; YAK = Yakima WA.

Culture independent assessment of the bacterial flora present in affected lung tissues of two bighorn sheep from each of the eight outbreak populations quantified the percentage of Pasteurellaceae-specific ribosomal 16S operons within the lung lesions: *P. multocida*, 12.7%; *B. trehalosi*, 8.7%; and *M. haemolytica*, 0.2% (Besser et al., 2008, 2012b) (Fig. 1). These data indicate that while these agents are frequently detectable in affected animals, they are relatively (*P. multocida*, *B. trehalosi*) or extremely (*M. haemolytica*) minor constituents of the infections. While this does not exclude the possibility that these agents may have been present in higher numbers in earlier stages of the disease, it is notable that their frequency did not differ between animals collected by gunshot early in the disease course and animals that died of the disease (Besser et al., 2012b).

3.2. Plausibility

As well established respiratory pathogens in many other animal hosts, Pasteurellaceae are generally plausible as causal agents of pneumonia in bighorn sheep. They also exhibit characteristics consistent with the long established association of bighorn sheep pneumonia with domestic sheep contact: bighorn sheep predominantly carry leukotoxin negative *B. trehalosi*, whereas most domestic sheep carry leukotoxin-expressing *M. haemolytica* (Sweeney et al., 1994; Jaworski et al., 1998). Therefore, the observed association of bighorn sheep pneumonia following domestic sheep contact is plausibly consistent with transmission

of leukotoxin expressing Pasteurellaceae, particularly *M. haemolytica*. On the other hand, given the highly epizootic nature of this disease, a causal agent isolated from multiple affected animals within an outbreak would be expected to be indistinguishable by appropriate molecular epidemiologic 'fingerprinting' genetic tests. In a few cases, indistinguishable strain types (or strains carrying unique artificial markers) have been demonstrated in bighorn sheep experimentally commingled with domestic sheep or goats, consistent with transmission (Rudolph et al., 2003; Kelley et al., 2007; Lawrence et al., 2010). However, in general, indistinguishable (epizootic) Pasteurellaceae strain types have not been identified either within natural outbreaks (Weiser et al., 2003; Besser et al., 2012b) or in outbreaks following experimental commingling of domestic sheep and bighorn sheep (Onderka and Wishart, 1988; Foreyt, 1990, 1994; Foreyt, 1998), calling into question Pasteurellaceae as epizootic disease agents.

3.3. Experimental evidence

The strongest evidence for the hypothesis that bighorn sheep pneumonia is caused by Pasteurellaceae is the rapidly lethal bronchopneumonia that frequently results from experimental challenge of bighorn sheep with leukotoxin positive *M. haemolytica* or *B. trehalosi* (Onderka et al., 1988; Foreyt et al., 1994; Kraabel et al., 1998). However, other experimental evidence does not unequivocally support this hypothesis. For example, commingling of bighorn

sheep and domestic cattle did not result in epidemic pneumonia (Foreyt, 1994; Foreyt and Lagerquist, 1996), despite the high prevalence of carriage by cattle of *lktA* positive *M. haemolytica* types A1 and A2, both highly lethal to bighorn sheep (Dassanayake et al., 2009; Foreyt and Lagerquist, 1996). Similarly, commingling bighorn sheep with deer or elk that were pharyngeal carriers of '*P. haemolytica*' or *P. multocida* did not result in any clinical disease over a six-month period (Foreyt, 1992). Furthermore, although sporadic pneumonia deaths attributed to *M. haemolytica* were observed in bighorn sheep experimentally commingled with non-ovine domestic ruminants carrying *M. haemolytica*, epizootic disease did not occur (Foreyt, 1994; Foreyt et al., 2009; Besser et al., 2012a).

It should be noted that *Pasteurella multocida*, a major respiratory pathogen of sheep, cattle, swine, and many non-ungulate species (Harper et al., 2011) and frequently isolated from bighorn sheep with pneumonia (Aune et al., 1998; Weiser et al., 2003; Rudolph et al., 2007), has as yet received relatively little research attention for its potential role in bighorn sheep pneumonia. *P. multocida* was frequently detected in pneumonic bighorn sheep involved in five of the eight outbreaks investigated by Besser et al. (2012b), was relatively uncommonly detected in lung tissues of healthy control animals (20%), and was the sole Pasteurellaceae for which some clonal strain types were observed within multiple animals within outbreaks (Besser et al., 2012b). To our knowledge, the effects of experimental challenge of bighorn sheep with *P. multocida* have not been reported. Therefore, more evidence is needed to critically assess the possible causal role of this bacterium in bighorn sheep pneumonia.

4. The role of *M. ovipneumoniae*

Initial observations of *M. ovipneumoniae* in bighorn sheep were made in pneumonic animals from the Black Mountains in Arizona in 1980 (Bunch et al., 1985). This agent was then reported in association with a pneumonia epizootic affecting Dall's sheep in a zoo (Black et al., 1988). Subsequently, *M. ovipneumoniae* was detected in animals removed from Hells Canyon during an epizootic in the mid-1990s (Rudolph et al., 2007). However, prior to development and widespread use of molecular detection techniques, *M. ovipneumoniae* received little attention, probably because its fastidious nature rendered it difficult to isolate in culture (Weiser et al., 2012).

4.1. Strength of association

M. ovipneumoniae is strongly associated with pneumonia in bighorn sheep. The strength of association of *M. ovipneumoniae* was first investigated in a study of bighorn lambs collected in Hells Canyon (Besser et al., 2008). In addition to documenting the presence of *M. ovipneumoniae* in all pneumonic lambs but not in a healthy lamb, the study also included a serosurvey of nine additional pneumonic bighorn sheep populations, all of which exhibited high seroprevalence, and nine healthy bighorn sheep populations, all of which were seronegative. Subsequently, *M. ovipneumoniae* was detected in pneumonic lung tissues of

>95% of 44 affected bighorn sheep lungs sampled in eight pneumonia epizootics that occurred in the western US during 2009–2010 but was absent in lung tissues of animals ($N=5$) obtained from two populations unaffected by pneumonia (Besser et al., 2012b). Table 1 lists evidence of *M. ovipneumoniae* presence as tested by culture, PCR, or specific serologic antibodies in bighorn sheep populations of known health status from across western North America. Exposure to *M. ovipneumoniae* was documented in all 36 populations tested that were classified as pneumonic by the wildlife officials submitting the diagnostic specimens, but in only 3 of 32 populations that were classified as healthy ($P<0.001$; odds ratio ∞ /undefined; 95% confidence interval = $31 - \infty$).

4.2. Temporality

As reported above, in recent years since reliable diagnostic tools have been available, we have not identified any bighorn sheep pneumonia outbreak in which *M. ovipneumoniae* was not detected in a high proportion of affected animals. In the retrospective serologic study reported in Besser et al. (2008), three populations were identified that remained seronegative up to the year preceding the first recognized pneumonia outbreak, following which all three populations developed high seroprevalence of *M. ovipneumoniae* specific antibody. Similarly, animals in the Asotin Creek population of Hells Canyon were repeatedly tested and remained seronegative and culture/PCR negative for *M. ovipneumoniae* prior to its first pneumonia outbreak in 2012, during which animals with pneumonia were PCR positive for *M. ovipneumoniae* and subsequent to which, surviving animals demonstrated seroconversion.

4.3. Plausibility

The biologic plausibility of a role for *M. ovipneumoniae* is addressed by several lines of evidence, including the role of a natural host (domestic sheep) as a risk factor for disease transmission, the nature of the lesions observed in affected bighorn sheep, and the molecular epidemiologic evidence of epizootic transmission of strains of this agent in disease outbreaks. The host range of *M. ovipneumoniae* is limited to Caprinae and the species colonizes domestic sheep flocks at high prevalence (Nicholas et al., 2008). As a result, domestic sheep, domestic goats, and mouflon are plausible biologic sources of this agent, whereas other ungulates, including domestic cattle, horses, llamas and wild Cervidae, are not. Purulent otitis media, a non respiratory lesion often observed in bighorn sheep with pneumonia (Besser et al., 2008), is also frequently associated with respiratory *Mycoplasma* infections in other hosts. Chronic bronchointerstitial pneumonia with lymphocytic cuffing of airways and bronchiolar hyperplasia are often observed in the lungs of bighorn sheep, typical of lesions observed in other respiratory *Mycoplasma* infections (Ettorre et al., 2007; Opriessnig et al., 2011). The lung histopathology of pneumonic bighorn sheep may also include acute fibrinous bronchopneumonia and pleuritis typical of Pasteurellaceae and different individuals from within the same outbreak may present with either

Table 1*M. ovipneumoniae* (Movi) status of bighorn sheep populations based on serology and/or culture/PCR.

Pneumonic populations			Non-pneumonic populations		
Population	State	Movi status	Population	State	Movi status
Kanab Creek	AZ	Positive	Cadomin	AB	Negative
E. Fraser Valley	BC	Positive	Ram Mt	AB	Negative
Peninsular	CA	Positive	Kofa	AZ	Positive
White Mts	CA	Positive	Navaho	AZ	Negative
Fossil Ridge	CO	Positive	Spence's Bridge	BC	Negative
Redbird	ID	Positive	Sierra Nevada	CA	Negative
L. Salmon River	ID	Positive	Wild Horse Island	MT	Negative
Morgan Cr	ID	Positive	Missouri Breaks	MT	Positive
Anaconda	MT	Positive	Sun River (to 2011)	MT	Positive
Bonner	MT	Positive	San Francisco River	NM	Negative
Cinnabar Basin	MT	Positive	Bare Mtns	NV	Negative
E.F. Bitterroot	MT	Positive	Montana Mtns	NV	Negative
Lower Rock Cr	MT	Positive	Monte Cristo Mtns	NV	Negative
Upper Rock Cr	MT	Positive	Muddy Mtns	NV	Negative
Sun River (since 2011)	MT	Positive	Pine Forest	NV	Negative
San Andreas	NM	Positive	River Mtns	NV	Negative
E. Humboldt	NV	Positive	Stonewall Mtns	NV	Negative
Hays Canyon	NV	Positive	Coglan Butte	OR	Negative
Pancake Range	NV	Positive	Imnaha (to 1995)	OR	Negative
Ruby Mtns	NV	Positive	Lostine (to 1986)	OR	Negative
Snowstorm Mtns	NV	Positive	Lookout	OR	Negative
Pilot/Leppy Hills	NV/UT	Positive	Badlands (south)	SD	Negative
Imnaha (since 1996)	OR	Positive	Clemans Mt	WA	Negative
Lostine (since 1987)	OR	Positive	Asotin Cr (to 2012)	WA	Negative
Sheep Mt	OR	Positive	Manson	WA	Negative
Muir	OR	Positive	Mt Hull	WA	Negative
Custer State Park	SD	Positive	Palmer Lake	WA	Negative
Hill City	SD	Positive	Quilomene	WA	Negative
Rapid Cr	SD	Positive	Sinlahekin	WA	Negative
Spring Cr	SD	Positive	Swakane	WA	Negative
Asotin Cr (since 2012)	WA	Positive	Tieton	WA	Negative
Black Butte	WA	Positive	Tucannon	WA	Negative
Wenaha	WA	Positive			
Yakima	WA	Positive			
Gros Ventre	WY	Positive			
Whiskey Basin	WY	Positive			

acute or chronic lesions or in some cases, the chronic lesion may be seen in different areas of the same lungs that exhibit the acute lesion (Besser et al., 2008; unpublished data). The inconsistent presence of acute lung lesions plausibly reflects the variable presence of secondary bacterial pathogens known to induce acute lesions (such as Pasteurellaceae), while the chronic lesions may reflect underlying *M. ovipneumoniae* disease. The pathophysiology of *M. ovipneumoniae* infection is believed to result from tracheal and bronchial ciliostasis and ciliary degeneration that diminish the ability of the infected animal to clear inhaled bacteria (Niang et al., 1998; Alley et al., 1999). The result of impaired mucociliary clearance is polymicrobial pneumonia, consistent with the diverse bacterial populations infecting lung tissues detected by the 16S studies described earlier (Fig. 1 and Fig. S1; Besser et al., 2008, 2012b). Finally, epizootic transmission of *M. ovipneumoniae* within bighorn sheep outbreaks has been confirmed by molecular epidemiology: indistinguishable 16S–23S intergenic spacer region DNA sequences were observed in animals within each of eight outbreaks, while strain types generally differed between outbreaks (Besser et al., 2012b).

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.prevetmed.2012.11.018>.

4.4. Experimental evidence

The role of *M. ovipneumoniae* in bighorn sheep pneumonia has also been tested experimentally both by exclusion and by introduction. As described earlier, previously published studies of bighorn sheep commingled with domestic sheep or mouflon reported very high (98%) pneumonia mortality within 90 days. In marked contrast and supportive of a key causal role for *M. ovipneumoniae*, three of four bighorn sheep survived commingling with *M. ovipneumoniae*-free domestic sheep for 100 days (Besser et al., 2012a). Experimental challenges of bighorn sheep with *M. ovipneumoniae* have produced more varied results. An isolate given to 1-week-old bighorn lambs failed to produce significant clinical disease: one of two challenged lambs developed a strong anti-*M. ovipneumoniae* antibody response and had focal chronic pneumonia but was culture negative at necropsy, while the other challenged lamb remained seronegative but culture positive from both the upper and lower respiratory tract through the >60 day experiment (Besser et al., 2008). Due to the possibility of in vitro attenuation of the *Mycoplasma* during the isolation process (Buddle et al., 1984), subsequent *M. ovipneumoniae* challenges by our laboratory have utilized nasopharyngeal washes collected directly from

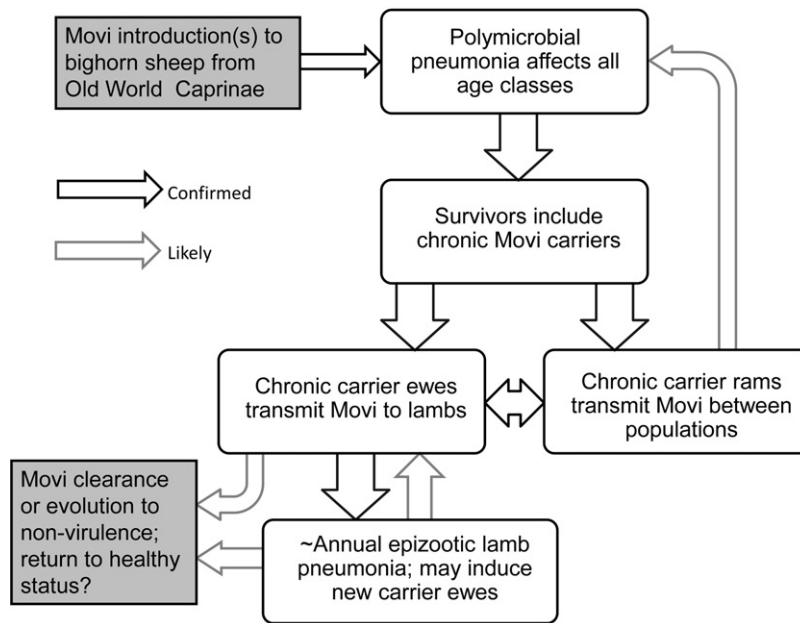


Fig. 2. Model diagram of the *M. ovipneumoniae* (Movi) hypothesis as presented in this paper.

M. ovipneumoniae colonized animals then treated by incubation (3 h, 35°C) with ceftazidime to eliminate Pasteurellaceae and other susceptible bacteria. These challenges have consistently produced signs of respiratory tract disease and pneumonia, including coughing, nasal discharge, ear droop, and head shaking similar to those seen in the natural disease. In one published study, four bighorns challenged in this manner developed clinical signs consistent with pneumonia, and one animal died during the 70 days that the animals were followed after challenge (Dassanayake et al., 2010). In subsequent experiments, transmission of *M. ovipneumoniae* infection from single infected animals to all in-contact animals and to all animals in adjacent pens was demonstrated in two separate experiments involving a total of nine bighorn sheep. All bighorn sheep infected by *M. ovipneumoniae* developed bronchopneumonia and several died (unpublished observations).

4.5. Analogy

Infection of swine with the closely related bacterium (*M. hyopneumoniae*) is similarly associated with 'atypical pneumonia' that results in a similar chronic lesion and that is similarly characterized by polymicrobial infection with a broad spectrum of inhaled oral and pharyngeal bacteria (Opriessnig et al., 2011).

5. Hypothesis: a conceptual model of etiology of bighorn sheep pneumonia

As reviewed earlier, among the many pathogens associated with bighorn sheep pneumonia, *M. ovipneumoniae* most completely satisfies accepted criteria for a causal role in disease, including strength of association, temporal

relationship, biological plausibility, experimental evidence and analogy (Rothman, 1986). These data suggest a new model (Fig. 2), in which a bacterium (*M. ovipneumoniae*) native to domestic sheep and goats but previously exotic to North American wild sheep, plays a key role: Epizootic all-ages pneumonia ensues when this agent is transmitted into a naive bighorn sheep population, as the bacterium interferes with normal bronchociliary clearance mechanisms (Niang et al., 1998) leading to polymicrobial pneumonia. The polymicrobial pneumonia frequently includes obligate anaerobic bacteria, Pasteurellaceae, *T. pyogenes*, and diverse other taxa. The case fatality rate of the pneumonia may be affected by the dose or the virulence of the infecting *M. ovipneumoniae* strain, the nature of the secondary bacterial invaders, the resistance of the host, or other as yet unidentified factors. Animals surviving the initial epizootic can become chronic carriers, shedding *M. ovipneumoniae* in the nasal secretions. Chronic carrier ewes may transmit the infection to subsequent years' lambs resulting in epizootic lamb pneumonia while chronic carrier rams may potentially spread the agent to adjacent populations in the course of their natural movements (Geist, 1971; Singer et al., 2000).

Once introduced into a bighorn sheep population, *M. ovipneumoniae* may be cleared after a year or two, or persist for decades. Potential ultimate outcomes of infection include selection for attenuation of the agent due to the high death loss of hosts infected with fully virulent strains, selection of bighorn sheep with increased genetic resistance to the infection, stochastic loss of carrier animals resulting in elimination of the agent and cessation of the infectious cycle, or local extirpation of bighorn sheep populations due to lack of lamb survival. Research is needed to better describe the range of

outcomes observed following bighorn sheep pneumonia and to determine which of the above mechanisms is at play.

If this hypothesized model is correct, efforts to control this disease by development or application of vectored vaccines to Pasteurellaceae are unlikely to provide significant beneficial effects, whereas efforts to ensure segregation of healthy bighorn sheep populations from *M. ovipneumoniae*-carrying domestic sheep, domestic goats, and chronically infected bighorn sheep are crucial to prevent new disease epizootics. In addition, it may be possible to develop *M. ovipneumoniae* vaccines or other management procedures that could reduce shedding of the agent by domestic small ruminants in order to reduce the risk of transmission to bighorn sheep. An effective vaccine could also potentially be usefully applied to bighorn sheep to reduce disease morbidity and mortality if the challenges inherent in vaccinating wildlife, particularly a species that inhabits inaccessible terrain, could be overcome.

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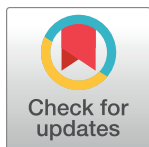
RESEARCH ARTICLE

Exposure of bighorn sheep to domestic goats colonized with *Mycoplasma ovipneumoniae* induces sub-lethal pneumonia

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Abstract

Background

Bronchopneumonia is a population limiting disease of bighorn sheep (*Ovis canadensis*) that has been associated with contact with domestic Caprinae. The disease is polymicrobial but is initiated by *Mycoplasma ovipneumoniae*, which is commonly carried by both domestic sheep (*O. aries*) and goats (*Capra aegagrus hircus*). However, while previous bighorn sheep comingling studies with domestic sheep have resulted in nearly 100% pneumonia mortality, only sporadic occurrence of fatal pneumonia was reported from previous comingling studies with domestic goats. Here, we evaluated the ability of domestic goats of defined *M. ovipneumoniae* carriage status to induce pneumonia in comingled bighorn sheep.

Methodology/Principal findings

In experiment 1, three bighorn sheep naïve to *M. ovipneumoniae* developed non-fatal respiratory disease (coughing, nasal discharge) following comingling with three naturally *M. ovipneumoniae*-colonized domestic goats. Gross and histological lesions of pneumonia, limited to small areas on the ventral and lateral edges of the anterior and middle lung lobes, were observed at necropsies conducted at the end of the experiment. A control group of three bighorn sheep from the same source housed in isolation during experiment 1 remained free of observed respiratory disease. In experiment 2, three bighorn sheep remained free of observed respiratory disease while comingled with three *M. ovipneumoniae*-free domestic goats. In experiment 3, introduction of a domestic goat-origin strain of *M. ovipneumoniae* to the same comingled goats and bighorn sheep used in experiment 2 resulted in clinical signs of respiratory disease (coughing, nasal discharge) in both host species. At the end of experiment 3, gross and histological evidence of pneumonia similar to that observed in experiment 1 bighorn sheep was observed in both affected bighorn sheep and domestic goats.

Conclusions/Significance

M. ovipneumoniae strains carried by domestic goats were transmitted to comingled bighorn sheep, triggering development of pneumonia. However, the severity of the disease was

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markedly milder than that seen in similar experiments with domestic sheep strains of the bacterium.

Introduction

Historical and contemporary field observations suggest that contacts with domestic sheep are followed by pneumonia outbreaks in previously healthy bighorn sheep populations [1–3]. Supporting these observations, >95% of 90 bighorn sheep in eleven studies involving contact with domestic sheep suffered fatal pneumonia within 100 days, as reviewed in [4]. However, the potential role of domestic goats in transmitting pneumonia pathogens to bighorn sheep is less clear, with relatively fewer observed bighorn sheep pneumonia outbreaks following domestic goat contacts [5–7] and with relatively little fatal bighorn sheep pneumonia observed in two experimental comingling studies with domestic goats (22% of 9 bighorn sheep) [5, 8]. Domestic goats may transmit respiratory pathogens when they contact bighorn sheep on or near bighorn sheep home ranges as stray animals from farmsteads, or when used for weed control, or when commercial operations are grazed on public lands in or near bighorn sheep home ranges, or when used as pack animals supporting back-country recreation. In addition, bighorn sheep may encounter domestic goats on private lands within or adjacent to bighorn ranges. Therefore, it is important to understand the risk that contact with domestic goats may pose for bighorn sheep health.

Mycoplasma ovipneumoniae, proposed as the primary pathogen that triggers bighorn sheep pneumonia outbreaks [9, 10] followed by persistently impaired recruitment resulting from recurrent lamb pneumonia [11, 12], has a host range limited to Caprinae [13]. Distinct host-specific clades of *M. ovipneumoniae* have recently been reported in domestic sheep and goats [7, 14]. In a Washington state survey of goat farms adjacent to bighorn sheep habitat, this pathogen was carried asymptotically by animals on 7 of 16 goat farms, and by 58% of individual goats on positive farms [15].

Like bighorn sheep pneumonia, respiratory disease of domestic sheep and goats is polymicrobial, frequently including *M. ovipneumoniae* (and perhaps also *Mycoplasma arginini*), *Mannheimia haemolytica*, *Pasteurella multocida*, and other pathogens. Chronic and persistent coughing is the main clinical sign observed associated with *M. ovipneumoniae* infection of domestic lambs, which is often described as summer pneumonia in Australia and New Zealand, or as coughing syndrome in the United States [13, 16]. Clinical severity ranges from mild illness associated with reduced growth rates to fatal polymicrobial pneumonia [13, 17]. *M. ovipneumoniae* has also been associated with outbreaks of severe respiratory disease in goats [13, 18, 19].

The *M. ovipneumoniae* carriage status of the animals used in previously published experimental domestic goat-bighorn sheep comingling studies was not defined [5, 8] and so it is possible that the low death rate reported in those studies was due to the absence of *M. ovipneumoniae* in the comingled goats. Here we tested the hypothesis that exposure to domestic goats carrying *M. ovipneumoniae* results in epizootic pneumonia in bighorn sheep by conducting a series of experimental comingling studies with domestic goats of defined *M. ovipneumoniae* carriage status.

Materials and methods

Ethics statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and in conformance with

United States Department of Agriculture animal research guidelines, under protocols #03793 and #04482 approved by the Washington State University Institutional Animal Care and Use Committee. As described in those protocols, euthanasia was to be performed by intravenous injection of sodium pentobarbital for animals observed to be in severe distress associated with pneumonia during the study and prior to any necropsy examination for surviving animals at the end of each experiment.

Experimental animals

Six healthy, *M. ovipneumoniae*-unexposed, pregnant adult bighorn ewes were provided by the Washington Department of Fish and Wildlife on January 25, 2014 from the Clemans Mountain population for these studies. This population had previously been confirmed to be uninfected and unexposed to *M. ovipneumoniae* as determined by repeated testing with both competitive enzyme-linked immunosorbent assay (cELISA) serology and nasal swab polymerase chain reaction (PCR) testing [20]. Blood and nasal swab samples collected from the six experimental animals on the day of capture were also negative by *M. ovipneumoniae* cELISA and PCR tests. The ewes were randomly allocated to two experimental groups of three animals each. Sampling or moving the bighorn sheep was conducted under anesthesia (butorphanol tartrate, azaperone tartrate, and medetomidine hydrochloride, reversed with atipamezole and naltrexone, BAM kit, Wildlife Pharmaceuticals, Inc. Windsor CO). Six domestic goats were purchased from three local private owners for use in the experiments. Domestic goats used in experiment 1 (group 1) included a 5+ year Saanen wether previously used as a pack animal, a 1 year old mixed breed wether intended for slaughter, and a 1 year old Boer wether from a herd used for weed and brush control. All were confirmed nasal carriers of *M. ovipneumoniae* based on PCR testing of nasal swab samples on at least two occasions prior to the study. Domestic goats used in experiments 2 and 3 (group 2) included one yearling Boer female and two yearling LaMancha wethers, all negative for *M. ovipneumoniae* by nasal swab PCR when purchased. Because they originated in a *M. ovipneumoniae*-positive herd, group 2 domestic goats were placed in isolation from other small ruminants and were serially re-tested for *M. ovipneumoniae* nasal carriage (5 times over a 4 week period) to confirm their negative status prior to the start of experiment 2.

Experimental design

Three experiments were conducted in which domestic goats (DG) of known *M. ovipneumoniae* status were comingled with susceptible bighorn sheep (BHS). In each experiment, comingled animals shared feed and water sources (pelleted hay supplemented with a corn/oats/barley mixture, free choice trace mineral salt, and a single frost-protected water source in each pen). Single roofed shelters were available in each pen, and plywood sheets covered the pen fences at the corners to provide windbreaks. All animals were observed daily.

Experiment 1. BHS group 1 (BHS29, BHS32, and BHS33; *M. ovipneumoniae*-negative) was comingled in a 372 m² pen (pen 1) with group 1 DG (DG1, DG2, and DG3; *M. ovipneumoniae*-positive) beginning February 18, 2014. During experiment 1, BHS group 2 (BHS27, BHS28, and BHS31; *M. ovipneumoniae*-negative) were housed in a separate 232 m² pen (pen 2) isolated >30 m from any other domestic or wild sheep or goats. All animals were observed closely for signs of respiratory disease for a 100 day period. On May 30, 2014, BHS group 1 animals were euthanized and subjected to complete necropsy examinations. DG group 1, having shown no clinical signs of respiratory disease during the experiment, were donated to the Washington State University veterinary teaching program.

Experiment 2. After group 1 animals were removed at the end of experiment 1, pen 1 was left empty for 40 days before BHS group 2 (*M. ovipneumoniae*-negative) was added on July 7,

2014. BHS group 2 composition was modified by the loss of BHS27 and by the addition of BHS28L (a lamb born to BHS28 on April 18, 2014 during the first experiment. On September 10, 2014, 65 days after BHS group 2 were placed in pen 1, DG group 2 (DG4, DG5, and DG6; *M. ovipneumoniae*-negative) was comingled to begin experiment 2, in which the comingled animals were again closely observed for signs of respiratory disease for a 100 day period.

Experiment 3. In experiment 3, a strain of *M. ovipneumoniae* obtained by nasal wash from a domestic goat (originating on a different farm than the source farms of DG groups 1 and 2) was introduced into the comingled BHS and DG from experiment 2, as follows: DG4 and DG5 were removed to an isolation pen where they were each inoculated with *M. ovipneumoniae* by instillation of 10 ml of ceftiofur-treated nasal wash fluids into conjunctivae and nasal passages. After confirmation of colonization by positive nasal swab PCR tests on days 3 and 7 following inoculation, they were re-commingled in pen 1 with *M. ovipneumoniae*-negative DG6 and BHS group 2 on February 16, 2015. The commingled animals were observed closely for signs of respiratory disease for a 100 day period. At the end of experiment 3, both BHS group 2 and DG group 2 were euthanized and subjected to complete necropsy examinations, as all had demonstrated signs of respiratory disease during the experiment.

Biosecurity

The study pens used for these experiments had not housed any BHS, DG or domestic sheep for >12 months prior to initiation of these experiments. Routine biosecurity measures during the experiments included broad physical separation (>1 km) and entirely separate animal care staff for pens 1 and 2. Animal care staff and researchers utilized personal clean protective equipment (coveralls, boots, and gloves) when entering study pens. Research staff occasionally entered both pens 1 and 2 on the same day, but if so always entered the *M. ovipneumoniae*-free pen 2 first, and changed personal protective equipment before entering pen 1.

Clinical scores

Clinical signs of respiratory disease were scored approximately 3 times per week using a point system: anorexia (1 point), nasal discharge (1 point), cough (2 points), nose licking (1 point), head shaking (1 point), ear paresis (1 point) and weakness/incoordination (1 point), as we have done previously [21].

Microbiological testing

Routine microbiological testing performed by the Washington Animal Diagnostic Laboratory (WADDL, accredited by the American Association of Veterinary Laboratory Diagnosticians), included detection of *M. ovipneumoniae*-specific antibodies in serum samples using competitive enzyme-linked immunosorbent assays (cELISA) [9, 22], detection of *M. ovipneumoniae*-specific DNA sequences by polymerase chain reaction (PCR) testing of nasal or bronchial swab eluates, culture-enriched swab samples (mycoplasma broth, 72 hrs, 35 C), or lung tissue samples [23, 24], detection of Pasteurellaceae in pharyngeal swab samples by conventional aerobic bacteriologic cultures and MALDI-TOF (Biotyper, Bruker, Woodlands TX) [25], and detection of neutralizing antibodies in serum samples indicating exposure to parainfluenza-3 and respiratory syncytial viruses by virus neutralization assays. PCR tests specific for detection of *M. haemolytica*, *B. trehalosi*, and *P. multocida*, and *lktA* (the gene encoding the principal virulence factor of *M. haemolytica* and *B. trehalosi*) were applied to DNA extracted from pneumonic lung tissues using previously described methods [26]. The 16S–23S ribosomal operon intergenic spacer (IGS) regions of *M. ovipneumoniae* recovered from animals in these studies

were PCR amplified and their DNA sequences determined in order to track transmission of specific *M. ovipneumoniae* strains (GenBank accession numbers pending) [7, 27].

Results

Experiment 1

All animals appeared clinically normal at the start of experiment 1. All BHS were negative for carriage (PCR testing of nasal swabs) and exposure to *M. ovipneumoniae* (cELISA testing of serum samples) (Table 1). DG were confirmed as carriers of *M. ovipneumoniae* based on detection of the bacterium by PCR on repeated nasal swab samples. Diverse pharyngeal Pasteurellaceae were detected in both BHS and DG during experiment 1 (Table 1). Group 1 BHS (BHS29, BHS32, and BHS33) and group 1 DG (DG1, DG2, and DG3) were commingled in pen 1 to begin the experiment, while group 2 BHS (BHS27, BHS28, and BHS31) were maintained in isolation in pen 2. Beginning 2–3 weeks after commingling, all group 1 BHS began showing signs of respiratory disease, including increased nasal discharge and coughing (Fig 1, S1 Table). Clinical signs of middle ear involvement (head shaking, ear droop) were rarely seen. After 70 days of illness, group 1 BHS exhibited decreasing clinical signs of respiratory tract disease, and group 1 DG remained clinically normal throughout the experiment. Group 1 BHS were euthanized and necropsied after 100 days of commingling. Uncommingled group 2 BHS remained healthy in pen 2 throughout experiment 1. At the end of experiment 1, the *M. ovipneumoniae*-negative status of group 2 BHS was re-confirmed by PCR and cELISA serology. BHS27 subsequently died from aspiration pneumonia, the onset of which followed the anesthesia conducted to permit sampling after experiment 1; at necropsy, BHS27 was also PCR- and ELISA-negative for *M. ovipneumoniae*.

All BHS ewes lambd during experiment 1. All lambs born to group 1 (goat-commingled, *M. ovipneumoniae*-positive) BHS died at less than 7 days of age, with signs of ocular, systemic and/or gastrointestinal tract disease (BHS29L, BHS32L, and BHS33L; Table 2). At necropsy,

Table 1. Microbiological status and pathologic lesions of animals in experiment 1.

Animal	Pen	Stage	Gross necropsy ¹	<i>M. ovipneumoniae</i> ²		<i>Pasteurellaceae</i> (<i>lktA</i>) ³
				PCR	cELISA (%I)	
BHS29	1	Pre	-	NotDet	NotDet (-12%)	P: Btre, Mhae+
		Post	LC (15% r; 5% l), CSE	Det: B, CS	Det (75%)	L: Pmul
BHS32	1	Pre	-	NotDet	NotDet (-13%)	P: Btre+
		Post	LC (5% l), CSE	Det: N, L	Det (60%)	L: NotDet
BHS33	1	Pre	-	NotDet	NotDet (3%)	P: Mhae+
		Post	LC (1% r) PA @ CSE	Det: B, N	Det (82%)	L: Psp
DG1	1	Both	-	Det: N	-	P: Btre, Mhae+
DG2	1	Both	-	Det: N	-	P: Mhae+
DG3	1	Both	-	Det: N	-	P: Btre, Mhae+
BHS27	2	Both	-	NotDet	NotDet (-7–3%)	P: Btre, Mhae, Psp
BHS28	2	Both	-	NotDet	NotDet (13–26%)	P: Btre, Mhae+, Pmul, Psp
BHS31	2	Both	-	NotDet	NotDet (7–16%)	P: Btre, Psp

¹ - = not done; LC = lung consolidation; CSE = cornual sinus exudate; PA = pleural adhesions; l = left lung; r = right lung.

²%I = Percent inhibition; NotDet = not detected; Det = detected; N = nasal passage swab; B = bronchus swab; CS = cornual sinus swab; L = lung tissue; — = not done.

³ Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; Btre = *Bibersteinia trehalosi*; Mhae = *Mannheimia haemolytica*; Pmul = *Pasteurella multocida*; Psp = *Pasteurella* species; + = *lktA* PCR positive; NotDet = No Pasteurellaceae detected.

<https://doi.org/10.1371/journal.pone.0178707.t001>

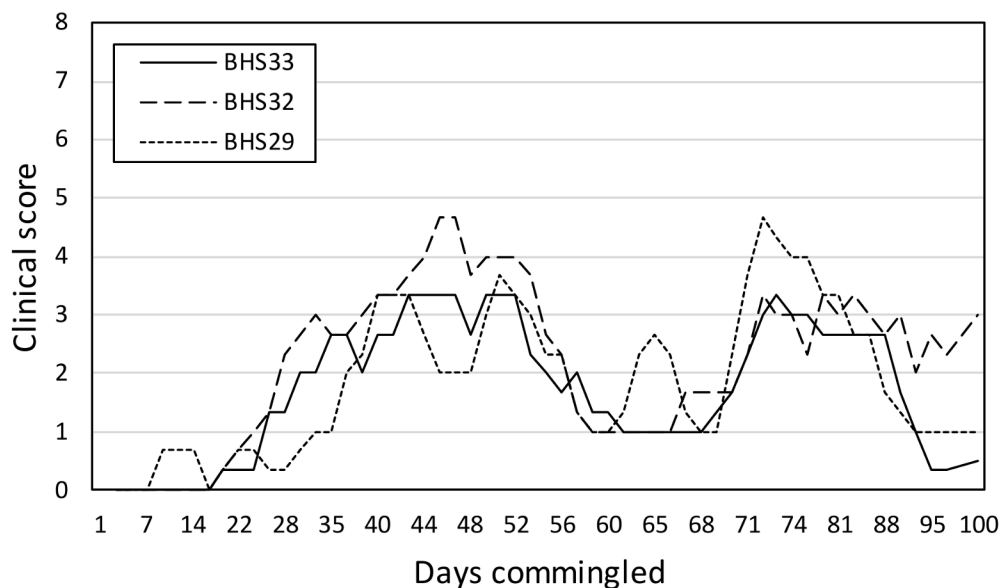


Fig 1. Clinical scores exhibited by bighorn sheep commingled with *M. ovipneumoniae* carrier domestic goats in Experiment 1. Three-day rolling average summed clinical scores based on observed anorexia, nasal discharge, cough, dyspnea, head shaking, ear paresis and weakness/incoordination. *M. ovipneumoniae* colonized DG were comingled with naïve BHS on day 0.

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none of these lambs had detectable pneumonia either grossly or histologically. The twin lambs born to group 2 (non-commingled; *M. ovipneumoniae*-negative) ewe BHS31 (BHS31L1 and BHS31L2) also died at 4 and 7 days of age, respectively, with signs of ocular, systemic and/or enteric disease. The lamb of group 2 BHS27 (BHS27L) was euthanized at 45 days of age necessitated by intractable diarrheal disease, while the lamb of BHS28 (BHS28L) survived.

Experiment 2

Group 2 DG, *M. ovipneumoniae*-free but carrying diverse pharyngeal Pasteurellaceae (Table 3), were comingled with group 2 BHS in pen #1. No respiratory disease signs were observed in either DG or BHS for >120 days during experiment 2.

Table 2. Outcomes and microbiological findings of lambs born during experiment 1.

Animal ID	Pen	Outcome ¹	<i>M. ovipneumoniae</i> ²		<i>Pasteurellaceae</i> (<i>IktA</i>) ³
			PCR	cELISA (% I)	
BHS29L	1	C (d6), death (d7)	Det: L N C	NotDet (0%)	P, L: Btre; C: Mhae+
BHS32L	1	C, D, death (d5)	Det: N	Det (50%)	P: Mhae+; C: Mhae+;
BHS33L	1	C, death (d7)	Det: N, B	Ind (49%)	P: Msp, Pmul; C: Mhae+; L: NotDet
BHS27L	2	D, euthanized d45)	NotDet	NotDet (2%)	NotDet
BHS28L	2	D (d54), survived	NotDet	NotDet (<4%)	P: Btre, Msp
BHS31L1	2	Death (d4)	NotDet	Not done	L: Mhae
BHS31L2	2	D, death (d10)	NotDet	NotDet (-9%)	L, C: Mhae+

¹C = conjunctivitis, D = diarrhea.

²%I = Percent inhibition; Det = detected; NotDet = not detected; Ind = indeterminate result; L = lung tissue; N = nasal passage swab; C = Conjunctival swab; B = Bronchial swab

³Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; C = conjunctival swab; Btre = *Bibersteinia trehalosi*; Mhae = *Mannheimia haemolytica*; Pmul = *Pasteurella multocida*; Msp = *Mannheimia* species; + = *IktA* PCR positive; NotDet = No Pasteurellaceae detected.

<https://doi.org/10.1371/journal.pone.0178707.t002>

Table 3. Microbiological status and pathologic lesions of animals in experiments 2 and 3.

Animal ID	Experiment	Gross necropsy ¹	<i>M. ovipneumoniae</i> ²		<i>Pasteurellaceae (IktA)</i> ³
			PCR ²	cELISA (%I)	
BHS28	Pre 2	-	NotDet	NotDet (26%)	P: Btre, Mhae+, Psp
	Post 2 Pre 3	-	NotDet	NotDet (12%)	P: Btre, Msp, Psp
	Post 3	PA (r, l)	Det: N, B	Det (79%)	P: Btre; L: Btre, Pmul
BHS28L	Pre 2	-	NotDet	NotDet (4%)	P: Btre, Mhae+, Psp
	Post 2 Pre 3	-	NotDet	NotDet (15%)	P: Msp
	Post 3	LC (5% r, 1% l)	Det: N	Det (83%)	P: Btre; L: Btre
BHS31	Pre 2	-	NotDet	NotDet (7%)	P: Btre, Psp
	Post 2 Pre 3	-	NotDet	NotDet (16%)	P: NotDet
	Post 3	No lesions seen	Det: N, B	Det (77%)	P: Btre; L: Btre, Psp
DG4	Pre 2	-	NotDet (x5)	NotDet (29%)	P: Btre, Mhae+
	Post 2 Pre 3	-	NotDet	NotDet (20%)	P: Btre
	Post 3	LC (10% r, 5% l)	Det: N, B	Det (79%)	L: Mhae
DG5	Pre 2	-	NotDet (x5)	NotDet (21%)	P: Btre, Psp+
	Post 2 Pre 3	-	NotDet	NotDet (21%)	P: Mhae
	Post 3	LC (5% r, 5% l)	Det: N, B	Det (55%)	P: Btre; L: Mhae, Btre
DG6	Pre 2	-	NotDet (x5)	NotDet (28%)	P: Btre; Mhae+, Psp+
	Post 2 Pre 3	-	NotDet	NotDet (27%)	P: Btre, Mhae
	Post 3	No lesions seen	Det: N, B	Det (63%)	P: Btre; L: Btre, Mhae

¹ - = not done, PA = pleural adhesions, LC = lung consolidation; l = left lung; r = right lung.

²%I = Percent inhibition; NotDet = not detected, Det = detected, N = nasal passage swab; B = bronchial swab.

³ Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; Btre = *Bibersteinia trehalosi*; Mhae = *Mannheimia haemolytica*; Pmul = *Pasteurella multocida*; Msp = *Mannheimia* species; Psp = *Pasteurella* species; + = *IktA* PCR positive; NotDet = No Pasteurellaceae detected by aerobic bacterial culture or by PCR.

<https://doi.org/10.1371/journal.pone.0178707.t003>

Experiment 3

Previously *M. ovipneumoniae*-free DG4 and DG5 began showing respiratory disease signs while still in isolation following *M. ovipneumoniae* inoculation and these signs continued after comingling in pen 1. All other pen 1 animals also developed signs of respiratory tract disease beginning shortly after re-comingling of DG4 and DG5 (Fig 2, S1 Table). The clinical respiratory disease scores of all animals in experiment 3 began diminishing after approximately 70 days of comingling. All animals were humanely euthanized for complete necropsy examinations at the end of experiment 3.

Necropsy findings

Gross necropsy examination of the bighorn sheep in experiments 1 and 3 and in the domestic goats in experiment 3 revealed limited areas of lung consolidation in most but not all animals (Tables 1 and 3). These areas were localized to the ventral tips or edges of the middle and anterior lobes, and were darker red in color and firmer on palpation than the adjacent normal lung tissues. In addition, several animals had strong fibrous adhesions between these same lung regions and the pericardial or parietal pleura, presumably representing resolved pleuritis. All animals in the study had similar histopathologic lesions that varied in severity, consisting of inflammation centered around bronchi and bronchioles and extending to include adjacent alveoli (Fig 3). Inflammation was characterized by peribronchiolar and perivascular lymphoid hyperplasia with secondary suppurative bronchiolitis and alveolar atelectasis.

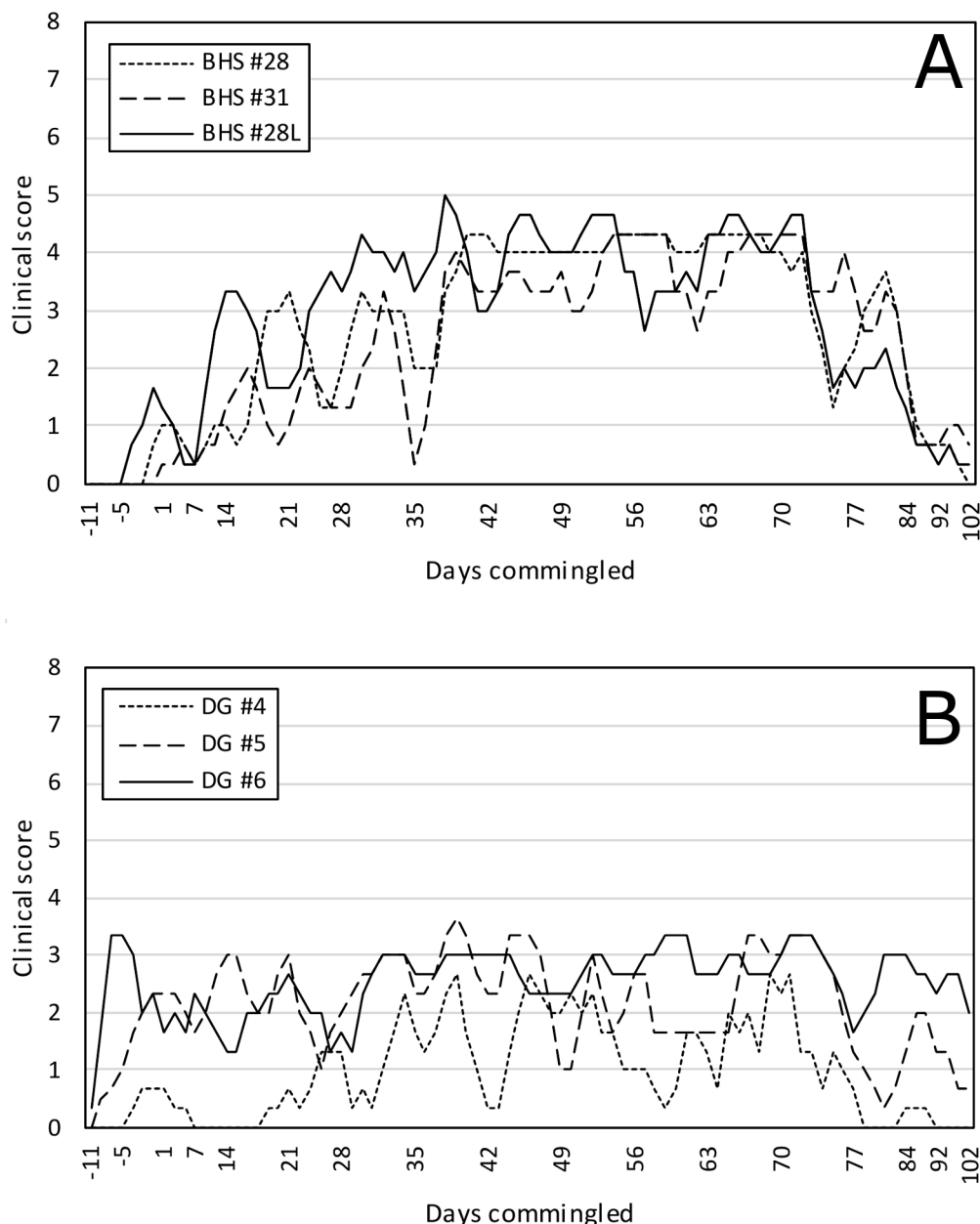


Fig 2. Clinical scores exhibited by commingled bighorn sheep and domestic goats in Experiment 3. Three-day rolling average summed clinical scores as described in Fig 1. (A) BHS; (B) DG. All BHS and DG in these experiments were naïve to *M. ovipneumoniae* prior to the experiment. DG4 and DG5 were exposed to *M. ovipneumoniae* on Day -11, and commingled with DG6 and BHS28, BHS28L and BHS31 on day 0.

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Microbiology findings

Microbiology and serology confirmed *M. ovipneumoniae* infection in all BHS only after contact with colonized DG, which resulted in seroconversion to this bacterium in both BHS and previously unexposed DG. *M. ovipneumoniae* IGS genotypes detected at necropsy in bighorn sheep or domestic goats shared identical sequences with those detected in the source goats prior to the studies. In experiment 1, a single IGS genotype was detected in all domestic goats prior to and after the experiment, and in all bighorn sheep at necropsy after the experiment. In

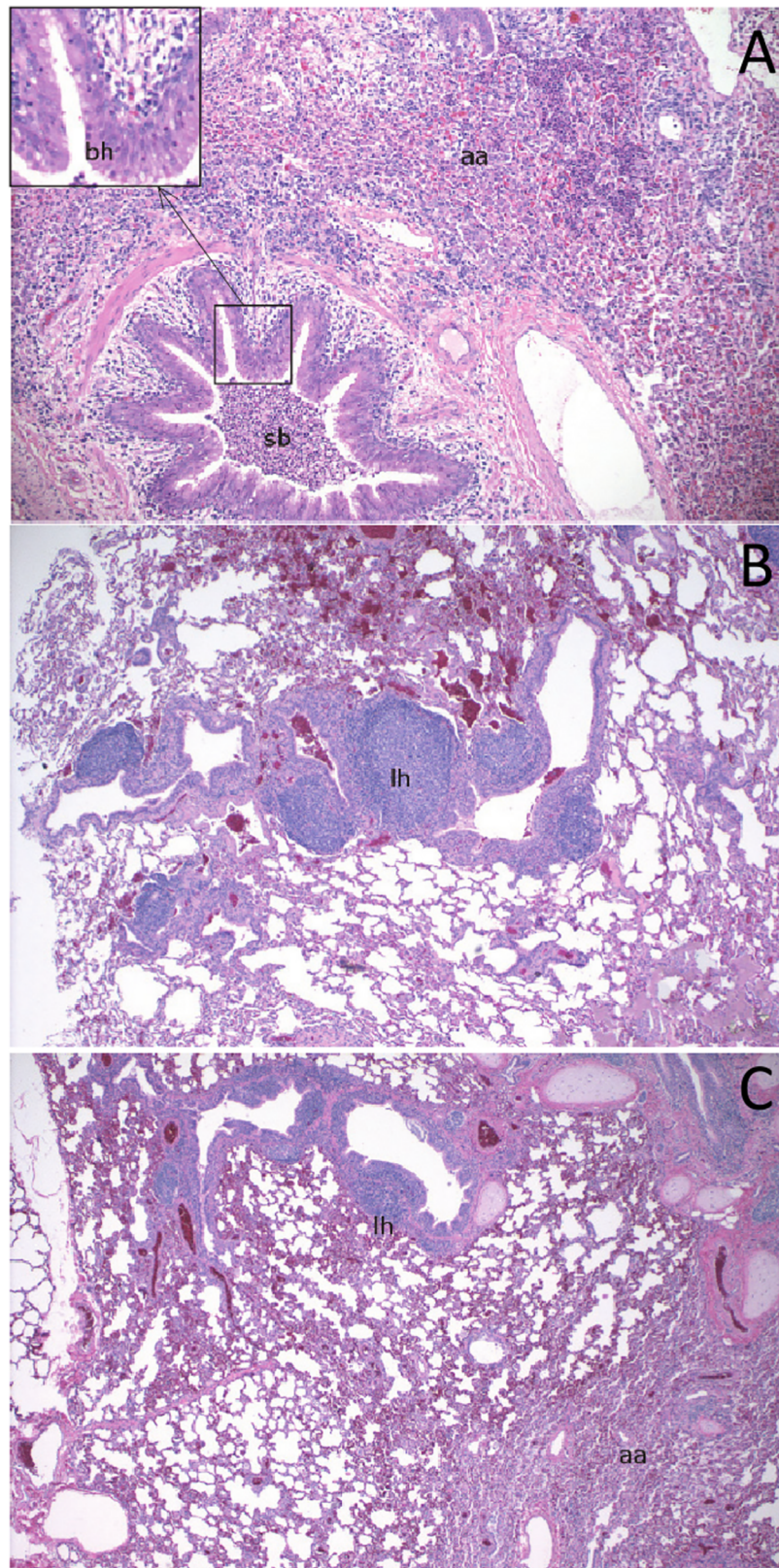


Fig 3. Representative histological lung lesions in experimental animals. Similar lesions were observed in all necropsied experimental animals. (A) BHS29 following experiment 1; (B) BHS28 following experiment 3,

and (C) DG5 following experiment 3. aa = alveolar atelectasis; bh = bronchiolar hypertrophy; lh = lymphoid hyperplasia; sb = suppurative bronchiolitis.

<https://doi.org/10.1371/journal.pone.0178707.g003>

experiment 2, two different IGS genotypes were detected in the domestic goat inoculum source, one of which was subsequently detected in all commingled goats and bighorn sheep, while the other strain was detected in all the domestic goats but was not detected in any of the comingled bighorn sheep. Both the bighorn sheep and domestic goat groups were colonized in the oropharynx by diverse Pasteurellaceae prior to, during, and after these experiments, and some of these bacteria were also isolated from pneumonic lung tissues of the animals at necropsy (Tables 1 and 3). These included *lktA*+ *M. haemolytica*, sometimes considered the most lethal Pasteurellaceae [23, 28].

Discussion

The results of this study both resemble yet substantially differ from those of previous studies of the impact of *M. ovipneumoniae* infection on initiation of pneumonia in bighorn sheep. Similar to previous studies exploring contacts with domestic sheep, comingling of *M. ovipneumoniae*-carrier domestic goats resulted in transmission of the pathogen to susceptible bighorn sheep, which then developed signs and lesions of respiratory disease [21]. However, contrary to previous studies that utilized domestic sheep carrying *M. ovipneumoniae*, the respiratory disease observed following experimental contact with domestic goats carrying *M. ovipneumoniae* was relatively mild, resulting in no fatalities. In addition, respiratory disease of comparable severity to that shown by the bighorn sheep also developed in naïve domestic goat hosts following infection, something that had not been reported in previous studies utilizing naïve domestic sheep. The lesions observed were consistent with those described in *Mycoplasma* spp. infections of sheep, goats and cattle, often called enzootic pneumonia. Enzootic pneumonia lesions are distinct from those caused by other bacterial agents of bronchopneumonia, such as *Pasteurella multocida* and *Mannheimia hemolytica*, which are characterized by widespread hemorrhage, necrosis and suppurative inflammation [13].

These contrasting results have interesting implications about the strain-specific virulence of *M. ovipneumoniae* and the specific roles of this and other bacterial pathogens in bighorn sheep respiratory disease. They also provide an area for additional research to identify management approaches for preventing new outbreaks of respiratory disease in bighorn sheep.

These results strengthen the previously documented links between exposure to *M. ovipneumoniae* and development of respiratory disease in bighorn sheep. All bighorn sheep exposed to goats carrying *M. ovipneumoniae* in experiments 1 and 3 developed signs and lesions of pneumonia, even when they had previously been exposed to the same goats in the absence of *M. ovipneumoniae*. All bighorn sheep kept in captivity under very similar conditions, but without contact with goats (experiment 1 pen 2) or in contact with *M. ovipneumoniae*-free goats (experiment 2, pen 1) did not develop signs of pneumonia. Pasteurellaceae bacteria carrying the gene encoding their principal virulence factor (*lktA* encoding leukotoxin) are another group of pathogens that have been hypothesized to play key roles in bighorn sheep pneumonia [23, 28], but both the bighorn sheep and the domestic goats used in these studies carried these pathogens prior to the experiments, and the presence of these pathogens were not clearly linked to the experimental outcomes or pathologic lesions.

Despite the consistent development of bighorn sheep pneumonia following contact with domestic goats carrying *M. ovipneumoniae*, the disease outcomes were more similar to those observed in the two previous experiments in which domestic goats were comingled with bighorn sheep and differed markedly from previous bighorn sheep-domestic sheep comingling

experiments [4, 5, 8]. Unfortunately, the *M. ovipneumoniae* carriage status of the goats used in those previous experiments was not tested and no clinical observation data were reported. The cumulative 98% mortality reported for bighorn sheep comingled with domestic sheep (88 deaths among 90 bighorn sheep in 11 experiments as reviewed in [4]) was significantly higher than either the mortality rates of those two previous goat commingling experiments (2-tailed Fisher's exact test, $P = 0.0014$ [5] and <0.0001 [8], respectively) or the two experiments reported here (each $P < 0.001$). Therefore, while bighorn sheep comingled with *M. ovipneumoniae* carrier goats consistently developed respiratory disease and pneumonia, mortality was a rare outcome compared to that observed when bighorn sheep were comingling with domestic sheep carrying *M. ovipneumoniae*.

One hypothesis that may explain the differing severity of outcome of bighorn sheep infected with *M. ovipneumoniae* originating from domestic goats is that *M. ovipneumoniae* with differing virulence traits may be harbored by these two host species. Emerging evidence supports the existence of distinct clades of *M. ovipneumoniae* from these two host species [7, 14]. A naturally occurring bighorn sheep pneumonia outbreak linked to *M. ovipneumoniae* belonging to the domestic goat clade, described in [7], resulted in approximately 30% pneumonia-associated mortality in the adult bighorn sheep, similar to that reported in [5] and towards the lower end of the range of mortality rates reported in pneumonia outbreaks affecting wild bighorn sheep [3, 29–32].

Since impaired recruitment due to *M. ovipneumoniae*-associated annual lamb pneumonia mortality is recognized as a key population-limiting effect of pneumonia in bighorn sheep [11], we anticipated that the lambs born to the ewes comingled with domestic goats in these experiments would provide insights into the effects of goat-origin *M. ovipneumoniae* on lambs. In both pens, lambs experienced a similar range of diseases but no bronchopneumonia was observed in any lamb, and the deaths in pen 1 occurred prior to the age at which lamb pneumonia typically is seen. Therefore, the experiments were not informative about lamb pneumonia resulting from *M. ovipneumoniae* carried by domestic goats, although the infections experienced by the pen 1 lambs clearly documented pathogen transmission among domestic goats, bighorn ewes, and bighorn lambs. Further studies will be needed to determine the persistent effects of goat-origin *M. ovipneumoniae* on bighorn sheep lambs in the years following initial exposure.

Conclusions and management implications: The severity of disease induced in bighorn sheep by the goat-origin *M. ovipneumoniae* strains in these experiments was relatively mild and transient. However, important questions remain: 1) Do other, more virulent strain-types exist within the goat *M. ovipneumoniae* clade? 2) Are goat-origin *M. ovipneumoniae* persistently carried by some bighorn sheep that recover from mild infections, as sheep-origin strains sometimes are? 3) If persistent carriers of goat-origin *M. ovipneumoniae* exist among bighorn ewes, are these strains subsequently transmitted to their lambs and do they induce lamb pneumonia? Answers to these questions will be relevant to the development of risk-appropriate management procedures to protect bighorn sheep from epidemic pneumonia.

Supporting information

S1 Table. Clinical scores of bighorn sheep and domestic goats.
(XLSX)

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Special Section on Mountain Sheep Management

Pneumonia in Bighorn Sheep: Risk and Resilience

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ABSTRACT Infectious disease contributed to historical declines and extirpations of bighorn sheep (*Ovis canadensis*) in North America and continues to impede population restoration and management. Reports of pneumonia outbreaks in free-ranging bighorn sheep following contact with domestic sheep have been validated by the results of 13 captive commingling experiments. However, ecological and etiological complexities still hinder our understanding and control of respiratory disease in wild sheep. In this paper, we review the literature and summarize recent data to present an overview of the biology and management of pneumonia in bighorn sheep. Many factors contribute to this population-limiting disease, but a bacterium (*Mycoplasma ovipneumoniae*) host-specific to Caprinae and commonly carried by healthy domestic sheep and goats, appears to be a primary agent necessary for initiating epizootics. All-age epizootics are usually associated with significant population declines, but mortality rates vary widely and factors influencing disease severity are not well understood. Once introduced, *M. ovipneumoniae* can persist in bighorn sheep populations for decades. Carrier females may transmit the pathogen to their susceptible lambs, triggering fatal pneumonia outbreaks in nursery groups, which limit recruitment and slow or prevent population recovery. The demographic costs of disease persistence can be equal to or greater than the impacts of the initial epizootic. Strain typing suggests that spillover of *M. ovipneumoniae* into bighorn sheep populations from domestic small ruminants is ongoing and that consequences of spillover are amplified by movements of infected bighorn sheep across populations. Therefore, current disease management strategies focus on reducing risk of spillover from reservoir populations of domestic sheep and goats and on limiting transmission among bighorn sheep. A variety of techniques are employed to prevent contacts that could lead to transmission, including limiting the numbers and distribution of both wild and domestic species. No vaccine or antibiotic treatment has controlled infection in domestic or wild sheep and to date, management actions have been unsuccessful at reducing morbidity, mortality, or disease spread once a bighorn sheep population has been exposed. More effective strategies are needed to prevent pathogen introduction, induce disease fadeout in persistently infected populations, and promote population resilience across the diverse landscapes bighorn sheep inhabit. A comprehensive examination of disease dynamics across populations could help elucidate how disease sometimes fades out naturally and whether population resilience can be increased in the face of infection. Cross-jurisdictional adaptive management experiments and transdisciplinary collaboration, including partnerships with members of the domestic sheep and goat community, are needed to speed progress toward sustainable solutions to protect and restore bighorn sheep populations. © 2017 The Wildlife Society.

KEY WORDS bighorn sheep, domestic goats, domestic sheep, *Ovis canadensis*, respiratory disease, spillover, wildlife-livestock interface.

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Infectious disease has influenced bighorn sheep (*Ovis canadensis*) population dynamics at least since the westward expansion of the United States, and plausibly since the Spanish colonization of Mexico and the American Southwest. The importance of disease in the historical decline and extirpation of bighorn sheep across much of their range from southern Canada to Mexico is unique among North American ungulates. Early naturalists described catastrophic die-offs and suggested that disease outbreaks and disappearance of wild sheep might be attributed to the introduction of domestic sheep and goats into bighorn sheep range (Brooks 1923, Grinnell 1928). Shillinger (1937) reported on an experiment in which Rocky Mountain bighorn sheep (*O. c. canadensis*), after surviving well in captivity by themselves, all died when healthy-appearing domestic sheep were introduced to the enclosure. Shillinger speculated “The only evident explanation is that some infectious organism well tolerated by the domestic sheep. . . was transferred to the wild animals with disastrous results” (Shillinger 1937:301). Since then, many more disastrous disease outbreaks have occurred in free-ranging wild sheep populations and another 12 domestic-wild sheep commingling experiments have been conducted with similar deadly results for bighorn sheep (Wehausen et al. 2011, Besser et al. 2012a). Together these observations have culminated in the recognition that management of bighorn sheep also involves management of pathogen transmission from domestic sheep (Council for Agricultural Science and Technology 2008, Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012, The Wildlife Society 2015).

The susceptibility of bighorn sheep to infectious agents carried by domestic sheep is not unexpected given that genetic similarity with domestic hosts is a key risk factor for pathogen spillover and associated disease-induced population declines in wildlife (Pedersen et al. 2007). The bighorn sheep is the only North American ungulate with a congeneric domesticated relative. Although species divergence occurred over a million years ago (Rezaei et al. 2010), domestic and bighorn sheep are still sufficiently similar that they can interbreed and produce viable offspring (Young and Manville 1960). Bighorn and domestic sheep and goats share lineages of immune-associated genes in the major histocompatibility complex (Gutierrez-Espeleta et al. 2001), but inherent differences in immune systems likely contribute to the disparity in effects of pathogens across species (Silflow et al. 1989, Dassanayake et al. 2009, Highland et al. 2016).

Understanding and acknowledging the importance of pathogen spillover from domestic sheep and goats has provided valuable perspective and direction for management of respiratory disease in bighorn sheep and, at the same time, has complicated it. Wildlife biologists managing bighorn sheep are now faced with an uncomfortable choice between promoting connectivity and gene flow to restore remnant populations and increasing fragmentation and limiting dispersal to reduce the risk of pathogen spillover and transmission. The impact of disease persistence in the aftermath of all-age epizootics is also a serious obstacle to population management. In this paper, we review the

literature and include a synthesis of data from our respective jurisdictions and from members of the Western Association of Fish and Wildlife Agencies Wild Sheep Working Group to provide an overview of the current state of knowledge about pneumonia in bighorn sheep. We report on impacts to individuals and populations, describe current management directions, and discuss potential strategies for moving forward.

CAUSES OF PNEUMONIA IN BIGHORN SHEEP

Pneumonia in bighorn sheep is a microbiologically complex disease, and many diverse bacteria are detected in the lungs of fatally affected animals, including pathogens that cause pneumonia and other diseases in livestock such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Fusobacterium necrophorum*. Some of these pathogens are toxigenic and lethal to captive bighorn sheep in experimental trials (Foreyt et al. 1994, Dassanayake et al. 2009), but they do not exhibit a clear and consistent association with disease epizootics in free-ranging populations (Singer et al. 2000b, Weiser et al. 2003, Rudolph et al. 2007, Besser et al. 2012b, Shanthalingam et al. 2016). Over time, paradigms of disease etiology have shifted, reflecting the diversity of pathogens and nonpathogenic agents detected in the lungs of pneumonic bighorn sheep. Suspected causes have ranged from lungworm infection (*Protostrongylus* spp.) to leukotoxin positive Pasteurellaceae, to a multi-factorial respiratory disease complex (Besser et al. 2013). Much attention has focused on virulent Pasteurellaceae bacteria where problems with accurate detection and classification have also complicated efforts to establish an association with pneumonia outbreaks in wild sheep (Angen et al. 2002, Walsh et al. 2012, Miller et al. 2013, Shanthalingam et al. 2014, Walsh et al. 2016).

In 2006, by applying culture-independent methods to high-quality samples of the lung microbiome obtained from free-ranging bighorn lambs in early stages of disease, researchers discovered that *Mycoplasma ovipneumoniae* was the pathogen that first invaded the lungs and predisposed affected animals to polymicrobial pneumonia (Besser et al. 2008). This pathogen does not act alone but appears to be a necessary agent for initiating epizootics. Further research is needed on the role of co-infection by known and perhaps as yet unrecognized pathogens as well as other factors that may contribute to disease outcomes by affecting transmission, carriage, and immunity (Dassanayake et al. 2010, Besser et al. 2012b, Fox et al. 2015, Wolff et al. 2016). Clarity on the significance of these interactions will help provide a more complete understanding of the variation observed in the course of infection and disease. We focus our discussion of microbial etiology on *M. ovipneumoniae* because, based on the experimental and empirical data which we review here, it currently presents the most parsimonious and well-supported model for a primary agent of bighorn sheep respiratory disease. For this reason it is also an important focus for management.

M. ovipneumoniae better meets Hill's (1965) causal criteria relevant to infectious diseases: strength of association,

temporality, plausibility, experimental evidence, and analogy than any competing proposed etiology (Besser et al. 2013). *M. ovipneumoniae* also fulfills Koch's postulates (Evans 1976, Walker et al. 2006) for a primary causal agent, with minor modifications. The strong association with disease (i.e., Koch's first postulate) is one of the most convincing lines of evidence for *M. ovipneumoniae*. Besser et al. (2013) detected *M. ovipneumoniae* in all free-ranging bighorn sheep populations affected by pneumonia epizootics where samples were available for testing ($n = 36$) and 91% (29/32) of bighorn sheep populations unaffected by pneumonia lacked evidence of exposure. Pneumonia outbreaks were associated with introduction of *M. ovipneumoniae* in ≥ 10 previously unexposed free-ranging bighorn sheep populations where testing was done before and after the epizootic (Besser et al. 2008, Bernatowicz et al. 2016; M. Cox, Nevada Department of Wildlife, unpublished data; J. Kanta, South Dakota Game, Fish, and Parks, unpublished data; J. Shannon, Utah Division of Wildlife, unpublished data; L. Jones, U.S. Fish and Wildlife Service, unpublished data). Limited information also suggests that free-ranging Dall's sheep (*Ovis dalli*) in Alaska and bighorn sheep populations in northern Alberta where bacterial pneumonia epizootics are not reported, have not been exposed to *M. ovipneumoniae* (Zarnke and Soren 1989, Besser et al. 2013).

Equally compelling, in 2 recent experiments 5 of 6 bighorn sheep survived when commingled with domestic sheep in the absence of *M. ovipneumoniae* (Besser et al. 2012a, Kugadas 2014). In contrast, virtually no (2%) bighorn sheep survived in 12 previous commingling experiments with domestic sheep, including only 1 of 26 in 4 experiments where presence of *M. ovipneumoniae* was reported or could be confirmed retrospectively (Foreyt and Jessup 1982; Foreyt 1989, 1990; Lawrence et al. 2010; Table S1, available online in Supporting Information).

Although Koch's second postulate (i.e., isolation of the agent in pure culture; Walker et al. 2006) has been repeatedly fulfilled, the ability of those cultures to reproduce the disease in healthy bighorn sheep (i.e., Koch's third postulate) is limited, perhaps because of virulence attenuation during cultural passage (Gilmour et al. 1979, Niang et al. 1998a, Besser et al. 2008). However, nasal washes from *M. ovipneumoniae*-colonized domestic sheep, treated to remove any detectable viable bacterial species other than *Mycoplasma*, do reproduce the disease in healthy bighorn sheep and the challenge strain of *M. ovipneumoniae* can be recovered from the pneumonic lungs of the affected animals, thereby fulfilling postulate 4 (i.e., re-isolation of the originally inoculated pathogen; Besser et al. 2014).

Many *Mycoplasma* spp. are host-specific, and the host range of *M. ovipneumoniae* is considered to be limited to Caprinae (Nicholas et al. 2008). Respiratory disease following infection with *M. ovipneumoniae* also has been reported in captive Dall's sheep and other wild Caprinae, including mountain goats (*Oreamnos americanus*), and muskox (*Ovibos moschatus*; Black et al. 1988, Handeland et al. 2014, Wolff et al. 2014). In a recent National Animal Health Monitoring System survey, Sheep 2011, the Animal and Plant Health

Inspection Service tested up to 16 adult females each in 453 randomly selected domestic sheep flocks from across the United States for *M. ovipneumoniae* nasal carriage and serum antibody. Most flocks (88%) tested positive for carriage (as determined by polymerase chain reaction [PCR] on nasal swabs). Larger operations were more likely to be PCR positive and all flocks with ≥ 500 adult females were PCR positive (USDA Aphis Veterinary Services 2015). Less extensive surveys of domestic goats reported 37.5–88% of flocks to be PCR positive on nasal swabs. Larger flocks were more likely to be positive for carriage (Heinse et al. 2016; Table S2, available online in Supporting Information). A host-specific pathogen commonly carried by domestic sheep and goats is consistent with the high mortality observed in captive bighorn sheep when commingled with domestic sheep but not when commingled with non-Caprinae livestock including cattle, horses, and llamas (Foreyt 1992, Foreyt and Lagerquist 1996, Besser et al. 2012a).

Additional evidence for *M. ovipneumoniae* as an epidemic agent is the transmission of 1 (or occasionally 2) multi-locus sequence types (strains) within an outbreak and a diversity of strains across outbreaks (Besser et al. 2012b, Cassirer et al. 2017). These strains of *M. ovipneumoniae* also link the all-age epizootics to the recurrent lamb pneumonia epizootics that follow (Cassirer et al. 2017). Strains detected in domestic sheep differ from those detected in domestic goats, suggesting host adaptation and coevolution within old world Caprinae (Maksimović et al. 2017). This divergence also provides a means for inferring the host species of origin.

CHARACTERISTICS OF RESPIRATORY DISEASE IN INDIVIDUALS

The diverse histopathologic lesions observed in experimental and naturally occurring bighorn sheep pneumonia, range from those typical of *Mycoplasma* infections (lymphocytic cuffing around airways and hypertrophy of the bronchial respiratory epithelium) to the often more dramatic and severe hemorrhagic, edematous, and necrotic lesions resulting from secondary bacterial infections (Miller 2001, Besser et al. 2008, Wood et al. 2017). This polymicrobial pneumonia is thought to occur when *M. ovipneumoniae* binds to and degrades the cilia of the trachea and bronchi, resulting in disruption of the mucociliary escalator (Niang et al. 1998c), the physiologic process for clearing bacteria from the lower respiratory tract. The impaired host immune defenses then allow inhaled opportunistic pathogens to establish multiple simultaneous infections of lung tissues with often fatal results.

The clinical course of bighorn sheep pneumonia may appear dramatic and short, but evidence from naturally occurring and experimental infection indicates that sub-clinical disease exists for several days to several weeks prior to development of obvious symptoms (Besser et al. 2008, Besser et al. 2014, Cassirer et al. 2017). This delay presumably represents the time required for *M. ovipneumoniae* to infect the airways and disrupt the mucociliary escalator. The latent period has important implications for management because animals might appear healthy for several weeks following

infection. By the time disease is evident, *M. ovipneumoniae* and other pneumonia pathogens already could be widespread in the population, even in individuals that still look healthy.

The original focus of *M. ovipneumoniae* research was infection in domestic sheep and goats where it is documented as an important, and probably under-diagnosed, cause of pneumonia in lambs and kids (Lin et al. 2008, Rifatbegović et al. 2011). Differences in the disease across host species suggest potential focal areas for research that may reveal why the disease is so devastating in wild sheep (Table 1). Higher nasal carriage rates ($\chi^2_1 = 35.49$, $P < 0.001$) and lower antibody prevalence in domestic sheep ($\chi^2_1 = 33.78$, $P < 0.001$; Table 1, Table S2) are consistent with an evolved tolerance of *M. ovipneumoniae*, defined as the ability to shed high levels of a pathogen with minimal morbidity or mortality (Råberg et al. 2009). Bighorn sheep resist infection and react to *M. ovipneumoniae* exposure with dramatic humoral immune responses, which could reduce carriage (Table 1), but also might trigger an auto-immune reaction. Such a reaction has been described in domestic lambs that develop respiratory disease associated with *M. ovipneumoniae* infection (Niang et al. 1998b). Robust bighorn sheep immune responses may also contribute to their disease.

Although *M. ovipneumoniae* may be associated with early pneumonia in domestic lambs (Bottinelli et al. 2017), juvenile domestic sheep are usually resistant to *M. ovipneumoniae* prior to weaning. Lambs born in persistently infected flocks often become infected during their third month of life (Table S3, available online in Supporting Information). Bighorn lambs are apparently completely susceptible to infection from birth (Besser et al. 2013), despite the similar magnitude and timing of passive transfer of maternal immunity in both species (Herndon et al. 2011, Highland 2016). Passively transferred bighorn sheep

antibodies might not protect from colonization or it could be that other forms of immunity are more important than the maternally transferred antibody-mediated immune response in defending the host from this pathogen (Plowright et al. 2013).

Domestic sheep herds usually harbor multiple strains of *M. ovipneumoniae* simultaneously (Thirkell et al. 1990; Ionas et al. 1991a, b; Parham et al. 2006). Therefore, intensive sampling and strain typing are required to confirm or rule out individual flocks as a source of *M. ovipneumoniae* transmission to bighorn sheep populations. In contrast, 1 or occasionally 2 strains appear to predominate in bighorn sheep populations (Cassirer et al. 2017). Immune response to *M. ovipneumoniae* is apparently strain-specific in both species, but disease outcomes of cross-strain infection are more severe in bighorn sheep (Alley et al. 1999, Felts et al. 2016, Justice-Allen et al. 2016, Cassirer et al. 2017).

PNEUMONIA IN BIGHORN SHEEP POPULATIONS

Die-Off Events

Many, if not most, bighorn sheep populations in the lower 48 states have endured all-age pneumonia die-offs (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012). These epizootics are the most obvious and dramatic manifestation of disease in bighorn sheep populations. During pneumonia outbreaks when animals are clinically ill, disease agents such as *M. ovipneumoniae* and Pasteurellaceae, usually transmitted through direct contact, may become airborne for short distances (Dixon et al. 2002, Besser et al. 2014). Pathogens can spread rapidly and expose nearly all individuals to infection (Bernatowicz et al. 2016, Ramsey et al. 2016, Cassirer et al. 2017). Severe, high mortality epizootics can ultimately cause extirpation or functional extinction of populations (Singer et al. 2000b); however, most pneumonia outbreaks do not kill entire populations. We estimated a median population decline of 48% (range = 5–100%) in 82 bighorn sheep disease events reported in 7 states and 2 provinces (Fig. 1, Table S4). Causes of the considerable divergence in mortality rates are not well understood but might be explained by heterogeneity in host immunity, pathogen virulence, and patterns of contact and transmission (Hobbs and Miller 1992).

We detected 28 different strains of *M. ovipneumoniae* in 45 bighorn sheep populations tested in 6 western states (Fig. 2B,C; Tables S5 and S6, available online in Supporting Information), each of which likely represents a separate spillover event that caused an all-age epizootic when first introduced. Domestic sheep and domestic goat *M. ovipneumoniae* lineages were both detected in bighorn sheep populations, but most strains detected in bighorn sheep fell within the domestic sheep clade (Kamath et al. 2016, Cassirer et al. 2017; Fig. 2C).

Clusters of the same strain in inter-connected populations, such as those along the border of Idaho, Oregon, and Washington in Hells Canyon, USA; in the Pancake Range

Table 1. Comparison of *M. ovipneumoniae* infection in domestic and bighorn sheep, USA, 1999–2016.

	Bighorn sheep	Domestic sheep
Infection outcome—naïve adults ^a	Bronchopneumonia	No disease
Infection outcome—lambs ^a	Bronchopneumonia 20–100% mortality	Coughing syndrome <2% mortality
Age of lambs at initial infection ^a	<1 week	Usually 8–12 weeks
Prevalence of carriage ^b	Low (median 22%)	High (median 56%)
Seroprevalence ^c	High (median 67%)	Low (median 30%)
Strain diversity within populations ^d	Usually 1	Usually many

^a Alley et al. (1999); Besser et al. (2008, 2014); Cassirer et al. (2013); USDA (2011).

^b Samples ($n = 1,267$) from 40 bighorn sheep populations in California, Idaho, Nevada, Oregon, Utah, and Washington and 47 domestic sheep flocks ($n = 2,508$ samples) in 13 states across the United States (USDA Aphis 2015).

^c Samples ($n = 1,589$) from 42 bighorn sheep populations in California, Idaho, Nevada, Oregon, Utah, and Washington and 37 domestic sheep flocks ($n = 323$ samples) across the United States (USDA Aphis 2015).

^d Parham et al. (2006), Cassirer et al. (2017).

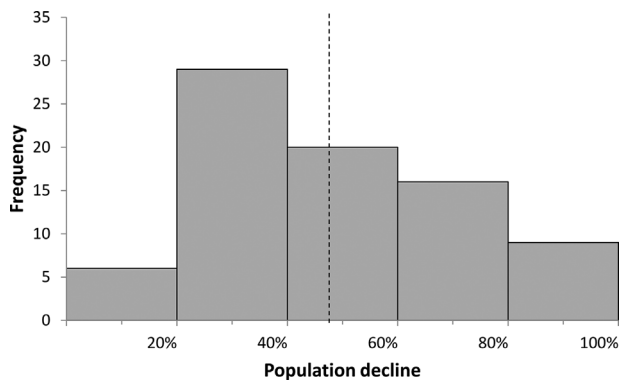


Figure 1. Population declines reported after pneumonia events in bighorn sheep populations in Alberta and British Columbia, Canada; and Idaho, Montana, Nebraska, Nevada, North Dakota, Oregon, Utah, and Washington, USA, 1978–2016. Dashed line represents median mortality of 48% in 82 pneumonia events. Data provided by state and provincial agencies and the Western Association of Fish and Wildlife Agencies Wild Sheep Working Group.

metapopulation of south-central Nevada, USA; and in the southern Nevada metapopulation (Fig. 2B), likely reflect a multiplier effect on a single spillover event when carrier bighorn sheep spread the pathogen across neighboring populations over time.

Multiple strains of *M. ovipneumoniae* observed within a single bighorn sheep population (Fig. 2B) often represent sequential pathogen invasion events. When a new strain is introduced into a population with ongoing infection, it may

replace the existing strain or eventually fade out. Retrospective analysis in the intensively sampled Hells Canyon metapopulation demonstrated a pattern of sequential spillovers and strain replacement or fadeout (Cassirer et al. 2017). Additional data and genomic analyses will be useful for confirming relationships among strains within and between populations and for more rigorous modeling of the ancestral phylogeny and transmission dynamics.

Pathogen Persistence

In 6 states (i.e., California, Idaho, Nevada, Oregon, Utah, and Washington), 63% of 155 populations where infection status is known, have been exposed to *M. ovipneumoniae*, including most native (never extirpated) herds (Fig. 2A). Exposure, as determined by the presence of *M. ovipneumoniae*-specific antibodies, indicates that at least some members of the population have been infected during their lifetime. Exposure does not confirm ongoing shedding, but infection is often maintained in exposed populations by (generally) asymptomatic carriers (Plowright et al. 2016, Cassirer et al. 2017).

Persistently infected populations have a high likelihood of prolonged periods of disease in juveniles and occasionally adults. High rates of pneumonia-induced lamb mortality (20–100%) between 4 and 14 weeks of age are common and reduce recruitment, limiting population growth or causing declines when combined with other mortality factors (Ryder et al. 1992, Enk et al. 2001, Smith et al. 2014, Smith et al. 2015). Some populations rebound (Coggins and Matthews

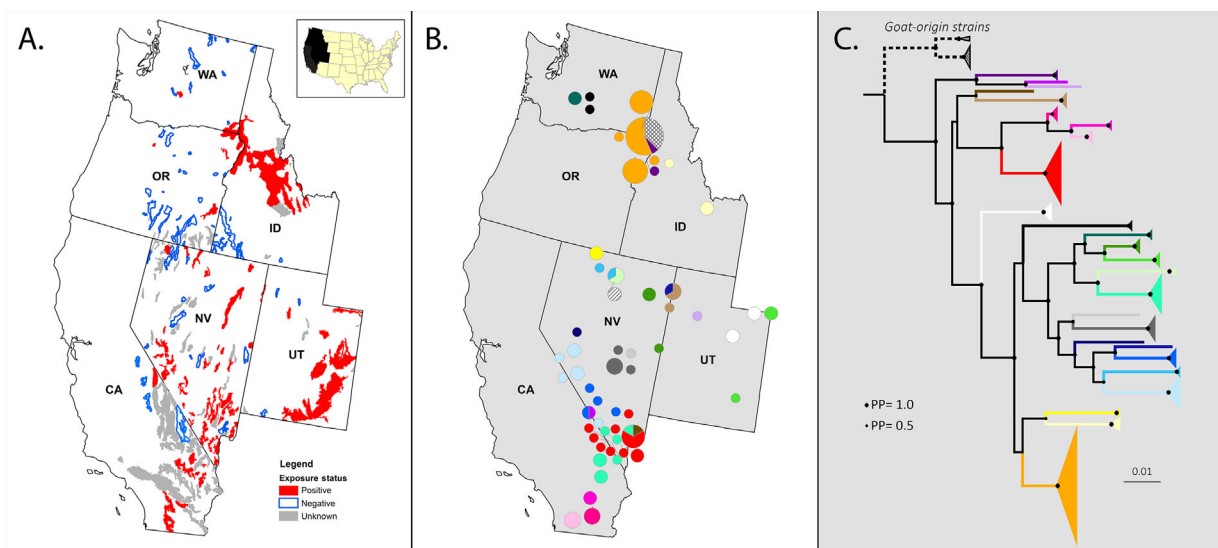


Figure 2. Exposure status and strain types of *Mycoplasma ovipneumoniae* detected in bighorn sheep populations in Washington, Oregon, Idaho, Utah, California, and Nevada, USA. A) Exposure of bighorn sheep populations to *M. ovipneumoniae*, 1999–2016. We assigned exposure based on results of blood serum samples ($n \geq 10$ from each population) submitted to the Washington Animal Disease and Diagnostic Laboratory for *M. ovipneumoniae*-specific serum antibody testing. Populations were classified as positive if antibodies were detected in at least one sample. Populations were classified as negative if no antibodies were detected from ≥ 15 samples. B) Spatial distribution of 28 *M. ovipneumoniae* genotypes (strains) obtained from bighorn sheep, 1984–2016. Each strain type is identified by a different color (domestic sheep origin, $n = 26$) or hatching (domestic goat origin, $n = 2$). Strains of the same color represent well-supported monophyletic clusters (posterior probability, PP > 0.95) with 99.7% to 100% sequence identity (as in panel C). In general, strains that are shades of the same colors are more closely related than strains of different colors. Pie charts indicate the proportional composition of strain types found at a given location over the entire time period of sampling. Chart size is relative to sample size. C) Bayesian majority rule consensus phylogeny of *M. ovipneumoniae* strains derived from bighorn sheep, with colors corresponding to strains shown in panel B. We based phylogenetic analyses on multilocus sequence typing data from 4 genetic loci (16S, IGS, *rpoB*, *gyrB*). Node support is reported as PP, with circle size relative to value (only PP > 0.60 shown). Scale bar indicates genetic distance in units of nucleotide substitutions per site. Dashed line represents the *M. ovipneumoniae* lineage derived from domestic goats.

1992, Jorgenson et al. 1997), but in others there is no trend towards recovery for decades (Manlove et al. 2016). In these cases, the demographic costs of pathogen persistence can outweigh the effects of the initial epizootic. Persistently infected populations also pose a disease risk for adjacent herds and, if they are used as source stock for translocations, moving carriers can inadvertently spread infection over long distances.

Chronically infected populations occasionally experience years with no evidence of disease in juveniles or adults. In approximately 20% of years following all-age disease epizootics in Hells Canyon, lamb survival was high and similar to that observed in unexposed populations (Cassirer et al. 2013). These sporadic disease fadeouts may be due to a delay or failure of *M. ovipneumoniae* transmission to susceptible lambs, as opposed to local pathogen extirpation, because pneumonia epizootics recur in subsequent years. A single or even several years with apparently healthy rates of lamb survival is not necessarily a harbinger of pathogen fadeout and population recovery (Manlove et al. 2016).

Social behavior likely plays an important role in determining the patterns of pneumonia epizootics and disease fadeout. Males are more likely to be directly associated with spillover and spread within and across populations during all-age outbreaks simply because they have larger home ranges and make more long distance movements (DeCesare and Pletscher 2006, O'Brien et al. 2014, Borg et al. 2016). However, dam-lamb and lamb-lamb interactions may be the most important routes of transmission in persistently infected populations (Manlove et al. 2017). Population substructure seems to protect some nursery groups from pathogens (Manlove et al. 2014) perhaps because no carrier dams are present. However, substructuring also might decouple contact rates and associated pathogen transmission from population size. If contact rates remain high as populations decline, transmission may never drop below the threshold required for pathogen extinction. This form of frequency-dependent transmission is common in social animals, and allows disease to persist at low population sizes. This can ultimately lead to host extirpation especially when combined with other stochastic events affecting small populations (de Castro and Bolker 2005).

MONITORING POPULATIONS FOR INFECTION AND DISEASE

All-age pneumonia epizootics are usually readily detected by observations of sick and dying sheep where populations are being actively monitored or are easily observed. However, low mortality outbreaks and epizootics in small and remote populations may be overlooked and underreported. Bighorn sheep also die from other diseases and not all sheep with clinical signs of respiratory disease (for example coughing) have pneumonia. Necropsy and laboratory testing are recommended when animals die from unknown causes, or when pneumonia is suspected. Pneumonia epizootics should be considered as a plausible cause when there is a sudden decline in a bighorn sheep population, particularly if followed by low recruitment.

Outside of all-age outbreaks, juvenile survival, particularly during the first 4 months of life, is the best demographic indicator of health status in bighorn sheep populations. Poor survival to weaning, (~4 months of age; Festa-Bianchet 1988), is the most sensitive signal of pneumonia-induced mortality in lambs. In Hells Canyon, there was a 100% probability of pneumonia being detected when survival to weaning was <50% (Cassirer et al. 2013, Manlove et al. 2016; Fig. 3A); however, this relationship might differ in areas with higher rates of non-disease-related neonatal mortality. Recruitment of juveniles as yearlings and population trend are less clear and specific metrics for classification of health status. Although most populations are stable or decline slowly during periods of persistent infection, pneumonia also might be present when lamb:female ratios at 9–12 months (recruitment) are ≤ 0.30 (Fig. 3A), even if populations are stable or slightly increasing (Fig. 3B, Table S7 available online in Supporting Information).

Diagnostic testing procedures for respiratory disease are continually changing as technology advances and knowledge of the disease and disease agents evolve. Comprehensive testing guidelines for wild sheep produced by the Western Association of Fish and Wildlife Agencies Wildlife Health Committee (2015) provide a good recent overview for a broad array of pathogens. Sampling for *M. ovipneumoniae* should be a part of any bighorn sheep health surveillance protocol and can also be used to monitor potential sources of domestic spillover. The most efficient diagnostic strategies for detection vary by host species and by infection stage. In acute infection (e.g., during all-age or lamb pneumonia epizootics in bighorn sheep, or in 8–16-week-old domestic lambs or kids in an enzootic flock), *M. ovipneumoniae* can be detected by PCR tests in a high proportion of animals' nasal swabs or pneumonic lung tissues. Infection status of domestic sheep and goat flocks is also best determined by PCR tests on nasal swabs. Given the high *M. ovipneumoniae* shedding prevalence in domestic sheep flocks (median 0.56; Table 1), PCR testing on swab samples from 10 adults should be sufficient (99% probability, binomial test) to detect whether *M. ovipneumoniae* is present. Repeated sampling is recommended to confirm negative status. In contrast, determining the exposure status of chronically infected bighorn sheep herds is most efficiently done by testing for serum antibodies, given the relatively high seroprevalence (median 0.67) and lower PCR prevalence in wild sheep (Table 1). Blood serum samples from 15 animals are generally adequate to determine exposure status if prevalence is ≥ 0.25 (99% probability, binomial test). If no antibodies are detected, the population can be considered unexposed, unless samples are collected recently after transmission, prior to immune response development. Nasal swabs from ≥ 18 animals should be adequate (85% probability) to detect shedding by PCR in bighorn sheep populations with *M. ovipneumoniae* prevalence of ≥ 0.10 . Larger sample sizes may be required to account for non-detection error associated with field sampling and diagnostic testing (Walsh et al. 2016). Strain-type can be identified in PCR-positive samples. Nasal or sinus swabs can also be collected from

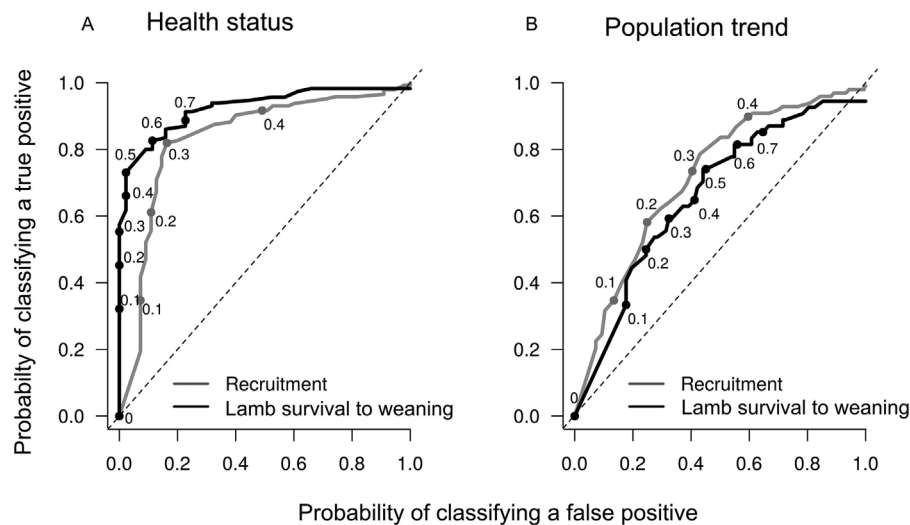


Figure 3. Receiver operating characteristic (ROC) curves identifying optimal lamb survival to weaning (black) and recruitment lamb:female ratios (gray) cut-off values for correctly classifying A) presence of pneumonia and B) declining population trend in bighorn sheep populations in Hells Canyon, Idaho, Oregon, and Washington, USA, 1997–2015 (Manlove et al. 2016). The y axis indicates sensitivity and the x axis indicates inverse specificity of lamb survival and recruitment values. A perfect predictor would have a value of 1 on the y axis and 0 on the x axis for a score of 1 AUC (area under curve). The dashed line represents values with no ability to predict categories (0.50 AUC). A) Lamb survival to weaning (AUC = 0.93) was an excellent predictor of health status and performed better than recruitment (AUC = 0.84). B) Recruitment (AUC = 0.72) was a better predictor of population trend than juvenile survival to weaning (AUC = 0.66). Optimal cut-off values for assigning presence of pneumonia were juvenile survival to weaning of <0.60 (84% accuracy) or recruitment of <0.30 lambs:female (82% accuracy). The optimal cut-off value for classifying populations as declining was recruitment of <0.20 lambs:female (69% accuracy).

fresh or frozen dead animals including heads of hunter-harvested bighorn sheep. Formalin-fixed paraffin-embedded pneumonic lung tissue blocks routinely archived by most diagnostic laboratories for histopathology also provide a DNA source for investigating historical presence and strain types of *M. ovipneumoniae* and other pathogens.

MANAGEMENT OF PNEUMONIA IN BIGHORN SHEEP

Wildlife managers and veterinarians have tried many techniques for controlling and mitigating respiratory disease in wild sheep populations, including administering antibiotics (Coggin 1988, Rudolph et al. 2007, McAdoo et al. 2010), vaccination (Cassirer et al. 2001, Sirochman et al. 2012), mineral supplementation (Coggin 2006, Sirochman et al. 2012), anthelmintic treatment (Miller et al. 2000, Goldstein et al. 2005), selective culling (Edwards et al. 2010, Bernatowicz et al. 2016, Ramsey et al. 2016), partial or complete depopulation (Cassirer et al. 1996, McFarlane and Aoude 2010, Bernatowicz et al. 2016), augmentation, and limiting population size and dispersal. The variety of methods employed, and the lack of clear successes, partially reflects past uncertainty over the causative agents and biological processes involved. The *ad hoc* nature of some of the treatments also limits broader inference. More rigorous testing of a broad ensemble of approaches for management is needed to account for the inherent challenges and variability associated with managing disease in free-ranging wildlife.

Large-scale pathogen eradication is rarely seen as a realistic goal (Klepac et al. 2013), particularly in the presence of a reservoir host, and indeed is not considered a viable option for wild sheep respiratory disease. Instead, more practical

management objectives include controlling the spatial extent or prevalence of the pathogen, facilitating natural pathogen extinction, or reducing the demographic costs of infection (Wobeser 2002, Joseph et al. 2013). Attempts to manage bighorn sheep pneumonia fall broadly into 2 categories: 1) strategies that directly aim to reduce exposure and transmission such as preventing spillover, treatment with antibiotics, vaccination, targeted culling of shedders, reducing population size or density, and population eradication; and 2) strategies that aim to increase individual resistance or herd resilience, including improving nutritional condition, increasing genetic diversity, managing co-infection, or increasing or modifying spatial structuring. Some approaches (such as vaccination or density reduction) could conceivably have application in both categories.

Preventing exposure, theoretically and in practice, offers the most direct and effective method for disease control. Managing transmission is a component of disease prevention strategies for most zoonoses and other spillover diseases (Ebinger et al. 2011, Viana et al. 2014) including test and cull for brucellosis, oral vaccination for rabies, and reduction of deer density for tuberculosis (Rupprecht et al. 1986, Schmitt et al. 2002, Slate et al. 2005, Schumaker et al. 2012). However, managing transmission can be a long-term and costly endeavor. Promoting individual resistance and population resilience has theoretical and empirical support in a number of systems. In general, managing populations to maximize their individual- or herd-level resilience makes good sense (Stephen 2014). Whether increased resistance and resilience can offset the costs of an exotic pathogen like *M. ovipneumoniae*, which generally produces high mortality rates in non-adapted but otherwise robust hosts, remains to

be seen. Below, we discuss past performance and future potential of these management strategies in combating bighorn sheep pneumonia.

Preventing Spillover and Pathogen Invasion

State and federal natural resource agencies have widely instituted policies to prevent pathogen spillover by encouraging or requiring spatial separation between wild sheep and domestic sheep and goats (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012, Bureau of Land Management 2016). Federal and state policies are informed by models, such as the USDA Forest Service's Bighorn Sheep Risk of Contact Tool (Woolever et al. 2015), which incorporate bighorn sheep space use, habitat preferences, foray probabilities, and demographics (Clifford et al. 2009, Cahn et al. 2011, Carpenter et al. 2014, O'Brien et al. 2014) to identify geographic locations with high risk of domestic-wild sheep contact. These models allow comparison of proposed management alternatives and assessment of population-level consequences for bighorn sheep. Resulting actions may take the form of closing or retiring public grazing allotments, altering their timing of use, trucking rather than trailing sheep between pastures, or changing grazing classification from domestic sheep or goats to other livestock (USDA Forest Service 2010, Bureau of Land Management 2017).

Other preventive management practices include capturing or culling escaped domestic sheep on bighorn sheep ranges and removing wild sheep observed near or commingling with domestics. Outreach efforts on private and public lands have encouraged landowner or public lands grazing permittee cooperation in double-fencing domestic sheep flocks in wild sheep habitat, using additional guard dogs, penning domestic sheep and goats at night, not turning sick sheep out to pasture, counting domestic sheep more frequently to better detect and gather strays, notifying local wildlife officials if wild sheep are observed near domestic sheep, and encouraging use of other best management practices (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012).

We are unaware of any formal evaluation of the success of existing separation strategies in preventing new outbreaks, though the regular appearance of new *M. ovipneumoniae* strains in bighorn sheep herds suggests there is room for improvement. Nevertheless, cross-species contact mitigation efforts almost certainly play a crucial role in reducing pathogen invasion. More work is needed to assess the strengths and weaknesses of existing approaches and to devise new and better strategies for managing both domestic and wild sheep to reduce transmission risk. Efforts are currently underway to investigate the feasibility of developing and maintaining *M. ovipneumoniae*-free domestic flocks, which could help reduce the significant risk of pathogen transmission from small domestic sheep and goat herds on private lands (Sells et al. 2015, Heinse et al. 2016, Cassirer et al. 2017).

Another paradigmatic approach to preventing pathogen introduction is reducing density of wild sheep populations. Associative studies (Monello et al. 2001, Sells et al. 2015)

report a positive relationship between wild sheep relative density (or population size) and risk of respiratory disease outbreaks. This relationship could mechanistically result from larger or higher density populations occupying a greater area and dispersing more widely or more often than smaller, lower density herds, with the consequence that increased density corresponds to increased contacts with neighboring domestic sheep or infected wild sheep herds (Monello et al. 2001). Evidence, however, for a density-dependent relationship in movements and dispersal in ungulates is limited and equivocal (Loison et al. 1999, Long et al. 2008) and pre-outbreak population sizes are often small (<50 to 200 animals) and do not differ from sizes of populations that remain healthy (Monello et al. 2001, Shannon et al. 2014).

Many reintroduced bighorn sheep populations experience robust or even exponential growth following initial establishment. When these populations are exposed to respiratory pathogens they often undergo die-offs followed by a prolonged period of low lamb recruitment, limiting recovery (Manlove et al. 2016). As a result, populations are often largest just prior to outbreaks, leading to a statistical, but not necessarily biological, association between population size and outbreak risk. The expected biological processes underlying a presumed density-dependent relationship are not evident, such as declining population growth rate or reduced juvenile recruitment (Jorgenson et al. 1997, Monello et al. 2001). Therefore, it is unknown whether reducing populations or keeping them small would actually mitigate risk, or whether disease outbreaks are simply associated with healthy, growing, and susceptible populations. Future work could pursue the underlying mechanisms directly and experimentally. Understanding the nature of observed associations of pneumonia and population size in bighorn sheep is needed to help minimize disease risk and maximize the number and distribution of wild sheep on the landscape.

Translocations have been widely and successfully used to increase the numbers, distribution, and genetic diversity of bighorn sheep populations (Singer et al. 2000a, Hogg et al. 2006, Olson et al. 2012). Translocations also present a clear risk for anthropogenically assisted pathogen introductions and opportunities for exposure at release sites (Cunningham 1996, Deem et al. 2001, Sainsbury and Vaughn-Higgins 2012, Aiello et al. 2014). Moving animals known to be positive for pneumonia pathogens into new ranges is risky (Western Association of Fish and Wildlife Agencies Wildlife Health Committee 2015). Mixing bighorn sheep from populations known to harbor pathogens with naïve animals, can and has, had poor results (Sandoval et al. 1987). Even if a pathogen is present in both source and recipient populations, immunity may not provide universal protection (Dassanayake et al. 2009, Cassirer et al. 2017). Most state, federal, and provincial agencies use health screenings to inform wild sheep translocation decisions. Careful matching of pathogen profiles, including relevant bacteria, viruses, and parasites in source, recipient, and adjacent bighorn sheep populations and selecting release sites with low risk of contact with domestic sheep and goats are important for translocation success. In practice, health surveys may be

conducted a year in advance at the herd level and with imperfect pathogen detection probabilities, resulting in uncertainty surrounding an individual's health status at the time of translocation. Furthermore, *M. ovipneumoniae* strain typing would not be expected to detect possible epitope variation resulting in immune escape, and health screenings are only as good as our knowledge of what to look for. Improved molecular-based approaches for detecting and describing pathogens and their associated virulence factors are needed. Development of rapid animal-side tests is in progress and, if successful, could also contribute to reducing disease risks posed by translocations.

Reducing Transmission During and Post-Epizootics

A number of agencies have attempted to manage active respiratory disease outbreaks. However, no management action, absent population eradication, has successfully stopped a pneumonia outbreak, and there is no evidence that any intervention has consistently reduced morbidity, mortality, or spread of disease. In part, this is due to the unplanned nature of outbreaks and the inability to randomly assign treatments and controls to matched populations to reliably test for an effect. Nevertheless, efforts to halt epizootics by administering antibiotic treatments (Sandoval et al. 1987, Coggins 1988, Rudolph et al. 2007, McAdoo et al. 2010), and by conducting random and selective culls (Cassirer et al. 1996, Edwards et al. 2010, Bernatowicz et al. 2016, Ramsey et al. 2016) have generally had mixed or negative results. In other wildlife species, depopulation has been successfully employed to prevent spread between populations, but culling zones or population segments to stop the spatial spread of epidemics have met with limited success (Wobeser 2002). Culling is rarely successful because by the time an epidemic is detectable, transmission is usually well under way; even if culling slows transmission, it is unlikely to stop it given imperfect detection of symptomatic animals, long infectious periods, ongoing contacts, and undetected animal movements within and between populations. Lack of success with antibiotics and vaccines administered during or after outbreaks may be a function of their low efficacy, targeting the wrong agent, or an inability to administer them appropriately in most free-ranging bighorn sheep populations.

Depopulation and reintroduction has occasionally been used in an attempt to manage small, particularly poorly performing herds struggling with persistent disease. Although this method may be effective when all members of the former herd are removed, significant effort is needed to ensure complete removal and that the ongoing risk of pathogen introduction is low. A current experimental management effort offering an alternative to depopulation of persistently affected populations exploits the relatively low shedding prevalence of *M. ovipneumoniae* in bighorn sheep by removing only chronic carrier females (Bernatowicz et al. 2016). The goal of this experiment is to stop the chain of transmission from dams to lambs and facilitate pathogen fade-out. If successful, this technique may be best applied to small, accessible populations, where extensive testing is

feasible, and the stochastic mortality of chronic carriers may bolster an active selective removal. In general, test-and-cull success hinges on test sensitivity, animal handling opportunity, pathogen prevalence, and the duration over which management is implemented. Targeted removal works best when a few individuals are responsible for most of the transmission (Lloyd-Smith et al. 2005, Streicker et al. 2013) and may require complete eradication of these carriers. For example, although test-and-cull efforts to control brucellosis in elk (*Cervus canadensis*) successfully met goals of reducing local prevalence (Scurlock et al. 2010, Schumaker et al. 2012) they never eradicated the disease, and upon the program's cessation, prevalence rapidly increased (Wyoming Game and Fish Department 2016). Test-and-cull strategies would ideally be timed to coincide with the lowest-possible pathogen prevalence and the highest levels of immunity, although we currently do not know when those minima and maxima occur.

Managing for Resistance and Resilience

Managing disease by maximizing individual resistance or population resilience has received renewed interest, particularly in the face of continuing challenges associated with direct control of transmission. Theoretical and empirical work across humans, domestic animals, and wildlife suggests that manipulating physiological condition, genetics, or co-infection can alter rates of morbidity and mortality and reduce infection intensity, which may in turn feedback on population-level dynamics (Beldomenico and Begon 2010). However, several studies have found that tradeoffs often exist between enhancing disease resistance and controlling transmission. For example, increasing food supply can minimize parasite-induced mortality (Pedersen and Greives 2008) but may also facilitate transmission (Becker et al. 2015), managing co-infections can reduce morbidity and mortality but can also accelerate pathogen spread (Ezenwa and Jolles 2015), and metapopulation structure can enhance disease spread while simultaneously allowing higher numbers of hosts to survive (Hess 1996, McCallum and Dobson 2002).

There are numerous examples of management actions intended to bolster individual resistance and overall population performance in struggling bighorn sheep populations but little systematic evaluation as to their efficacy. For example, there is no clear evidence of a causal relationship between nutritional condition and susceptibility to respiratory disease in bighorn sheep. Certainly the many experiments in captivity show that optimally provisioned bighorn sheep still succumb at high rates upon exposure to respiratory pathogens. Disease resistance may be correlated with genetic diversity (Luikart et al. 2008, Savage and Zamudio 2011) and researchers continue to seek evidence of host genetic resistance to respiratory disease, which might be expected in herds that are demographically successful even in the presence of long-term pathogen persistence but, to date, a genetic basis has not been found for the susceptibility of wild sheep to pneumonia (Gutierrez-Espeleta et al. 2001, Boyce et al. 2011). Currently, multi-jurisdictional efforts are

underway to collect data on animal condition, genetics, and pathogens to better understand their interactions with wild sheep health.

At the population level, maximizing resilience might include promoting large, widely distributed, genetically diverse metapopulations with spatial structuring and a range of behaviors (de Castro and Bolker 2005, Hess 1996). Indeed, there is some evidence that larger wild sheep populations may experience lower rates of mortality during pneumonia epizootics and are more able to recover than their smaller counterparts (Singer et al. 2001, Cassaigne et al. 2010). Furthermore, increasing population substructure may create asynchrony in transmission across groups of animals. Although this may not prevent epizootics and could actually increase pathogen persistence at the herd or metapopulation level (Grenfell and Harwood 1997, Swinton et al. 1998, Park et al. 2002), it might buffer against simultaneous population-wide epizootics and facilitate stochastic pathogen extinction from sub-herd or population segments (Cross et al. 2005). More work is needed to determine whether or not spatial structuring shields bighorn sheep populations from the worst outcomes of disease and how population structure might affect disease persistence. Current efforts are underway on a limited basis to expedite formation of metapopulation structure by assisted colonization of adjacent range. These manipulations may be most applicable to large, healthy populations.

MANAGEMENT IMPLICATIONS

The extensive costs of pathogen introduction and transmission observed across a wide range of habitats and populations indicate that preventing spillover is the most pressing immediate priority for management of pneumonia in bighorn sheep. Collaboration by wildlife and livestock managers on research and in practice is needed to develop more effective, sustainable approaches to reduce ongoing pathogen transmission from domestic small ruminants to wild sheep. Transmission risks posed by moving bighorn sheep to expand populations are also recognized and should be mitigated before translocations are conducted. In the absence of spillover, selection on the host and the pathogen may eventually lead to a less destructive relationship between wild sheep and the bacteria involved in pneumonia. However, considerable theory suggests that evolution toward increased resistance or reduced virulence is not always expected (Alizon et al. 2009, Osnas et al. 2015). Effective tools are needed to actively restore persistently infected stagnant or declining populations. A comprehensive examination of disease dynamics across populations to better understand how recovery occurs naturally would be useful to inform management of pneumonia in exposed populations.

In the long-term, agencies will need better strategies for the management of larger interconnected bighorn sheep populations for species viability. Engaging a diversity of perspectives in the wildlife, domestic animal, and health sciences through an inter- or trans-disciplinary process could provide new directions or refine existing approaches for management of healthy, resilient populations (Choi and Pak 2007, Allen-Scott et al. 2015). Natural

experiments and designed experiments conducted in an adaptive management framework can also accelerate learning about complex natural systems (Walters and Green 1997, Craig et al. 2012, Williams and Brown 2016). Inter-jurisdictional collaboration can greatly facilitate and, in many cases, is required for successful adaptive management. Replicated interventions with clear hypotheses, objectives, and defined expected outcomes accompanied by monitoring of treatments and controls could greatly advance understanding in the face of uncertainty and speed progress towards developing successful strategies for managing pneumonia in wild sheep.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

Risk of Pathogen Spillover to Bighorn Sheep from Domestic Sheep and Goat Flocks on Private Land

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ABSTRACT Bighorn sheep (*Ovis canadensis*) across North America have suffered population losses due to polymicrobial pneumonia typically initiated by spillover events of bacteria from domestic sheep and goats. Because vaccination or treatment of individual animals remains an elusive goal and pneumonia often persists in bighorn herds for years or decades following infection, preventing contact between domestic and wild animals is widely accepted as the best prophylactic. For the past decade, most management efforts have focused on the risks associated with commercial sheep grazing on public lands; less attention has been paid to risks to bighorns from small flocks of domestic sheep and goats managed entirely on private land. We surveyed owners of 40 sheep or goat flocks located near bighorn sheep herds in central and southeastern Washington, USA during 2014 and 2015, to better understand their knowledge level, management practices, and willingness to reduce risks. Over one-third of sheep or goat owners had no knowledge of the potential for pathogen spillover to bighorns, but all were interested in reducing risk of interacting with bighorns, particularly by options that did not restrict their autonomy. We also sampled nasal mucosae of 137 animals in 24 flocks for presence of *Mycoplasma ovipneumoniae*, the bacterium most closely associated with bighorn pneumonia. *M. ovipneumoniae* was detected in 37.5% of sheep or goat flocks sampled, and animals had escaped their enclosures in 78% of these. Physical contact, and thus pathogen spillover from domestic sheep or goats living in small, private flocks in close proximity to bighorns is clearly a risk. We provide recommendations to agency staff on identifying, prioritizing and testing small herds, and then working with owners to reduce the risk of pathogen spillover.

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KEY WORDS bighorn sheep, disease, domestic goats, domestic sheep, landowner attitudes, *Mycoplasma ovipneumoniae*, *Ovis canadensis*, pneumonia.

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Evidence for strain-specific immunity to pneumonia in bighorn sheep

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Evidence for Strain-Specific Immunity to Pneumonia in Bighorn Sheep

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ABSTRACT Transmission of pathogens commonly carried by domestic sheep and goats poses a serious threat to bighorn sheep (*Ovis canadensis*) populations. All-age pneumonia die-offs usually ensue, followed by asymptomatic carriage of *Mycoplasma ovipneumoniae* by some of the survivors. Lambs born into these chronically infected populations often succumb to pneumonia, but adults are usually healthy. Surprisingly, we found that introduction of a new genotype (strain) of *M. ovipneumoniae* into a chronically infected bighorn sheep population in the Hells Canyon region of Washington and Oregon was accompanied by adult morbidity (100%) and pneumonia-induced mortality (33%) similar to that reported in epizootics following exposure of naïve bighorn sheep. This suggests an immune mismatch occurred that led to ineffective cross-strain protection. To understand the broader context surrounding this event, we conducted a retrospective analysis of *M. ovipneumoniae* strains detected in 14 interconnected populations in Hells Canyon over nearly 3 decades. We used multi-locus sequence typing of DNA extracts from 123 upper respiratory tract and fresh, frozen, and formalin-fixed lung samples to identify 5 distinct strains of *M. ovipneumoniae* associated with all-age disease outbreaks between 1986 and 2014, a pattern consistent with repeated transmission events (spillover) from reservoir hosts. Phylogenetic analysis showed that the strain associated with the outbreak observed in this study was likely of domestic goat origin, whereas strains from other recent disease outbreaks probably originated in domestic sheep. Some strains persisted and spread across populations, whereas others faded out or were replaced. Lack of cross-strain immunity in the face of recurrent spillovers from reservoir hosts may account for a significant proportion of the disease outbreaks in bighorn sheep that continue to happen regularly despite a century of exposure to domestic sheep and goats. Strain-specific immunity could also complicate efforts to develop vaccines. The results of our study support existing management direction to prevent contacts that could lead to pathogen transmission from domestic small ruminants to wild sheep, even if the wild sheep have previously been exposed. Our data also show that under current management, spillover is continuing to occur, suggesting that enhanced efforts are indicated to avoid introducing new strains of *M. ovipneumoniae* into wild sheep populations. We recommend looking for new management approaches, such as clearing *M. ovipneumoniae* infection from domestic animal reservoirs in bighorn sheep range, and placing greater emphasis on existing strategies to elicit more active cooperation by the public and to increase vigilance on the part of resource managers. © 2016 The Wildlife Society.

KEY WORDS bighorn sheep, disease ecology, Hells Canyon, livestock-wildlife interface, molecular epidemiology, multi-locus sequence typing, *Mycoplasma ovipneumoniae*, *Ovis canadensis*.

Pneumonia in bighorn sheep (*Ovis canadensis*) is a population-limiting disease associated with transmission of pathogens from domestic sheep and goats (Foreyt and Jessup 1982, Besser et al. 2013, Cassirer et al. 2013). As with many other wildlife diseases, a lack of comprehensive, system-specific information hampers disease management (Besser

et al. 2013, Joseph et al. 2013). Limiting contact with domestic sheep and goats is the primary strategy for preventing disease emergence in bighorn sheep (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012, The Wildlife Society 2015). Although currently the best management option available, implementation is politically and logistically difficult because of the natural movements of wild animals, unregulated presence of domestic sheep and goats on private lands, and concerns by the livestock industry about losing access to public grazing allotments. Reliable knowledge about health

threats and potential solutions will help wildlife and land managers make appropriate risk management decisions that will succeed in resolving the problem of pneumonia in bighorn sheep.

Based on the hypothesis that *Mannheimia haemolytica* expressing leukotoxin is the key causal pathogen, researchers have tested numerous vaccines to boost immunity to disease in bighorn sheep. However, so far no vaccine has protected wild sheep commingled with domestic sheep or goats in captive settings or shown potential for efficacy in free-ranging animals (Callan et al. 1991, Kraabel et al. 1998, Cassirer et al. 2001, Subramaniam et al. 2011, Sirochman et al. 2012). There may be several reasons for the elusiveness of an effective vaccine. First, there is a basic question as to the role of *M. haemolytica* in the disease (Besser et al. 2013) and second there are significant technical difficulties associated with vaccine development and application. Experimental challenge with leukotoxin-positive *M. haemolytica*, a well-described respiratory pathogen in domestic ruminants, is lethal to bighorn sheep in captivity (Foreyt et al. 1994, Dassanayake et al. 2009). However, *M. haemolytica* is only weakly associated with pneumonia epizootics in free-ranging bighorn sheep populations (Besser et al. 2012b). The pathology, microbiology, and the course of disease experimentally induced with *M. haemolytica* also do not match observations from the field (Besser et al. 2014). Recently, application of sensitive molecular diagnostic techniques on high quality samples led to identification of *Mycoplasma ovipneumoniae*, a previously overlooked bacterium, as the pathogen most strongly supported as a primary causal agent of pneumonia in bighorn sheep (Besser et al. 2008, 2012a, b).

M. ovipneumoniae is host-specific to Caprinae, and is frequently carried asymptotically by domestic sheep and goats (Martin and Aitken 2000). When introduced into naïve bighorn sheep populations, outbreaks of polymicrobial pneumonia ensue, sometimes resulting in high mortality in all age classes (Besser et al. 2008, 2014). After all-age pneumonia outbreaks, surviving adults usually maintain good health and normal life spans, although some individuals chronically carry *M. ovipneumoniae* in their upper respiratory tract (Besser et al. 2013). Both carriers and non-carriers are resistant to disease although this protection fails to prevent epizootics in lambs (Plowright et al. 2013, Manlove et al. 2016).

M. ovipneumoniae is also associated with mild and transient respiratory disease, usually in juveniles, in its normal domestic sheep and goat hosts (DaMassa et al. 1992, Martin and Aitken 2000). However, several investigators have reported that *M. ovipneumoniae* infections in domestic sheep and goats can cause severe pneumonia, particularly when multiple strains are present (Parham et al. 2006, Rifatbegović et al. 2011). This could be linked to a strain-specific immune response that fails to provide universal protection. Many pathogens are able to evade host immune responses by expressing a diversity of surface-exposed targets for neutralizing antibodies. From influenza virus to *Mycoplasma* spp., antigenic variation within and across strains enables

immune escape by pathogens and also complicates development of vaccines (Citti et al. 2010, Vink et al. 2012, Quiñones-Parra et al. 2014).

We documented the effects of invasion of a novel strain of *M. ovipneumoniae* into a group of free-ranging bighorn sheep that had harbored adult carriers for nearly 20 years following an all-age pneumonia outbreak (Cassirer et al. 1996, Plowright et al. 2013). Our expectation was that these adults were immune to the pathogen and that invasion of a new strain would not cause disease.

STUDY AREA

We conducted this study near Heller Bar at the mouth of the Grande Ronde River in Asotin County, Washington, USA (46.079° N, -116.986° W). The area was located in low elevation (250–1,250 m) canyon grasslands and cliffs along the breaks of the Grande Ronde and Snake Rivers on the northern edge of Hells Canyon. Summers were hot ($\bar{x}$ highs in Jul and Aug = 26–32° C) and winters were mild ($\bar{x}$ lows in Dec and Jan = -2 to 2° C). Average annual precipitation was 31 cm. July and August were the driest months and peak precipitation occurred in May. Plant associations were dominated by perennial bunchgrass (*Pseudoroegneria spicata* and *Festuca idahoensis*) communities, with deciduous riparian shrub stringers and upland shrub-fields. Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*) stands occurred on northerly aspects. In addition to bighorn sheep, common ungulates in the study area included mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and elk (*Cervus elaphus*). Potential predators of bighorn sheep included cougars (*Felis concolor*), bobcats (*Lynx rufus*), coyotes (*Canis latrans*), wolves (*C. lupus*), and black bears (*Ursus americanus*). Over 50% of the area was publicly owned and managed by federal and state agencies chiefly for wildlife, recreation, and seasonal (spring) cattle grazing. A low density, unincorporated rural community was scattered on adjoining private rangelands at the mouth of the Grande Ronde River and along the adjacent Snake River.

Following extirpation in the early 1900s, the Washington Department of Fish and Wildlife reintroduced bighorn sheep to the Joseph Creek Wildlife Area near Heller Bar. Between 1977 and 1989, 39 sheep were translocated from Washington, Oregon, and Montana to establish the Black Butte population (Johnson 1995). This became one of 16 interconnected populations that comprise the Hells Canyon bighorn sheep metapopulation. The Black Butte population increased to approximately 215 animals before a pneumonia outbreak occurred in 1995; 70% of the sheep died or were transferred to captivity in an attempt to stop the epidemic (Cassirer et al. 1996). The source of the outbreak was thought to be domestic sheep or goats on private lands within the Black Butte bighorn sheep population range (Rudolph et al. 2003). The population never recovered because of chronically low recruitment due to pneumonia-induced mortality in lambs (Plowright et al. 2013). By 2013, only 36 bighorn sheep were observed in surveys, and the population was estimated at 45 (Cassirer et al. 2013, Washington Department of Fish and Wildlife, unpublished data). Three

spatially distinct female groups occur in the population: Heller Bar, Shumaker, and Joseph Canyon. The groups are connected by movements of males, but we observed no female interactions across groups during this study. The Heller Bar female group was most accessible from the road and was the subject of this investigation.

METHODS

Observations

We monitored the Heller Bar female group during 2013 and 2014 as part of a study of contact patterns and lamb survival. We could individually identify 4 (31%) and 11 (85%) of the 13 adult females in 2013 and 2014, respectively, by numbered ear tags and color-coded and numbered very high frequency (VHF) radio-collars. One unmarked female was missing a horn, so all 13 sheep were individually identifiable in 2014. We located marked animals from the ground by radio-telemetry and then observed them through binoculars and a spotting scope. We conducted frequent and intensive observations between 1 May and 16 July to document productivity and neonatal survival (2013 median observation interval = 4 days, median duration of each observation = 3 hr; 2014 median observation interval = 1.5 days, median duration = 2 hr). Frequency of observation from 17 July through 26 August was every 10 days in 2013 and every 5 days in 2014, and from 26 August through the first week in October we observed the sheep once a month in 2013 and every 10 days in 2014. Median duration of observations from 17 July through October was 1 hour in both years. At each observation we recorded female and lamb health and behavior. Animals observed with nasal discharge, droopy ears, head shaking, or lethargy received a clinical score of 1, and animals observed coughing received a clinical score of 2. Sheep with no evidence of disease received a clinical score of 0.

Radio-collars on adults were equipped with a switch that triggered a fast pulse mortality signal if no movement was detected for 4 hours. We conducted site investigations, and where possible, retrieved carcasses whole when mortalities were detected. Where this was not possible, we conducted field necropsies and collected the head, the respiratory tract, and grossly abnormal tissues when available. We detected lamb mortalities through observation and retrieved whole dead lambs when autolysis was not too advanced for diagnostic testing (Cassirer and Sinclair 2007). We assigned lamb mortality dates as the midpoint between the last live observation and either the date when the carcass was found, or the date when the dam was first observed without a lamb if no carcass was located. We assumed a female had lost her lamb when it was found dead or when the number of lambs declined and she was never again observed with a lamb that year. All cadavers and tissues were submitted to the Washington Animal Disease and Diagnostic Laboratory (WADDL; Pullman, WA, USA) for analysis.

Health Sampling

We captured and sampled females in February ($n = 11$ of 13) and July ($n = 2$ of 11) 2014. In October 2014, we resampled

all 8 remaining sheep when we transferred them to captivity. We conducted captures via helicopter netgun and by darting from the ground with chemical immobilizing agents. All capture and handling followed animal care protocols approved by the Washington Department of Fish and Wildlife. Sampling entailed collecting throat swabs and placing them in buffered glycerol or Port-a-cul transport media (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA, #220144, #221606) for aerobic culture to detect Pasteurellaceae, swabbing nasal passages and placing swabs in mycoplasma broth (#102, Hardy Diagnostics, Santa Maria, CA, USA) for culture enrichment and polymerase chain reaction (PCR) detection of *M. ovipneumoniae* (Ziegler et al. 2014), and collection of serum for detection of antibodies to *M. ovipneumoniae* (competitive enzyme-linked immunosorbent assay [ELISA]), bovine respiratory syncytial virus (virus neutralization [VN]), bovine virus diarrhea (VN), infectious bovine rhinotracheitis (VN), and bovine parainfluenza-3 (PI-3, VN). Diagnostic testing on the above samples was conducted by WADDL.

We also collected upper respiratory washes by flushing nasal passages with 50 ml of phosphate-buffered saline and swabbed the oropharynx. We kept samples cool and processed them within 48 hours of collection, or stored them at -20°C . We extracted DNA (DNeasy, Qiagen, Redwood City, CA, USA) from swabs, from 10-ml aliquots of nasal wash, and from lung tissues of animals that died during the study as well as 2 pneumonic lambs that died in adjacent populations in 2013 to test for presence of pneumonia agents. We used a multiplex PCR to detect Pasteurellaceae including *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mannheimia* spp. (Besser et al. 2012b) and performed PCR for *lktA*, the gene encoding leukotoxin A, the major virulence factor of *Mannheimia* spp. and *B. trehalosi* (Walsh et al. 2016). If an agent was detected by either PCR or culture on any sample, we classified the animal as positive for that agent.

Strain Typing

Health of the Hells Canyon bighorn sheep metapopulation has been intensively monitored since the 1995 pneumonia outbreak in the Black Butte population, and intermittently prior to this. Therefore, we had access to fixed, frozen, and fresh lung tissue and swab samples collected from 1995–2015 in the Heller Bar female group and from 1986–2015 in adjacent female groups and populations. We extracted DNA from a subset of these sources to detect and strain type *M. ovipneumoniae* within the study population and the metapopulation through time. Detection was based on conventional (McAuliffe et al. 2003) and realtime (Ziegler et al. 2014) PCR.

We used multi-locus sequence typing (MLST) to characterize strains using partial DNA sequences of the 16S–23S intergenic spacer region (IGS), the small ribosomal subunit (16S), and the genes encoding RNA polymerase B (*rpoB*) and gyrase B (*gyrB*). We amplified these targets with PCR using a suite of existing and newly developed primers (Table 1). Because of the high degree of DNA sequence

Table 1. Oligonucleotide primers for polymerase chain reactions (PCR) used to amplify *Mycoplasma ovipneumoniae* multi-locus sequence typing (MLST) targets. Nesting refers to external and internal primer sets used for nested PCR reactions for amplification of the MLST loci, IGS, *rpoB*, and *gyrB*, when amplification from the default (internal) primers produced insufficient DNA template for sequencing.

Target ^a	Nesting	Oligonucleotide primer sequence	Reference
LM-F		TGAACGGAATATGTTAGCTT	McAuliffe et al. (2003)
LM-R		GACTTCATCCTGCACTCTGT	McAuliffe et al. (2003)
Ex-IGS-F	External	GTTAACCTCGGAGACCATTG	This paper
Ex-IGS-R	External	GTTTGCTAGGTTGGGTTTCC	This paper
IGS-F	Internal	GGAACACCTCCTTTCTACGG	Besser et al. (2012b)
IGS-R	Internal	CCAAGGCATCCACCAAATAC	Besser et al. (2012b)
Ex- <i>rpoB</i> -F	External	AGTTATCACAATTTATGGATCAAA	This paper
Ex- <i>rpoB</i> -R	External	GCTCAAAGTTCCATTTCCNCCGAA	This paper
<i>rpoB</i> -F	Internal	TCGGCTTCAGCAATTCCTTTCTT	This paper
<i>rpoB</i> -R	Internal	TCGGCTGTTGGGTTGTCTTCTC	This paper
Ex- <i>gyrB</i> -F	External	AAAACGWCCAGGKATGTATATTGG	This paper
Ex- <i>gyrB</i> -R	External	GGATCCATTGTTGTTTCTCATAATTG	This paper
<i>gyrB</i> -F	Internal	GGGTCAAACAAAAGCAAACTAAA	This paper
<i>gyrB</i> -R	Internal	ACGGAATAAAATGTCAAAAGTAA	This paper

^a LM = small ribosomal subunit (16S) gene, IGS = 16S–23S intergenic spacer, *rpoB* = β subunit of RNA polymerase gene, *gyrB* = gyrase B gene, F = Forward, R = Reverse.

variation in *M. ovipneumoniae*, the biggest challenge in developing this ensemble of loci was identification of targets with sufficiently conserved primer binding site sequences to enable consistent PCR amplification for subsequent sequencing. The loci selected for this study each exhibited extensive sequence polymorphism and together provide a highly discriminatory test. We typed the concatenated sequences using multi-locus sequence analysis because of the large numbers and diversity of the alleles detected at each target locus. We aligned sequences using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>, accessed 06 Sep 2016). We report sequence divergence as the percentage non-identity.

All PCR protocols included a preliminary dissociation phase (95°C, 15 min), amplification, and a final extension phase (72°C, 7 min). We amplified the 16S target using 35 cycles of 30 seconds each of dissociation (95°C), annealing (58°C), and extension (72°C). We amplified the IGS target using 35 cycles of dissociation (95°C, 1 min), annealing (52°C, 2 min), and extension (72°C, 2 min). For nested IGS amplification, the parameters for the external primers were identical except that the annealing temperature was 54°C. For *rpoB* and *gyrB*, we used inner and nested reactions with identical amplification conditions: dissociation (95°C, 1 min), annealing (50°C, 1 min), and extension (72°C, 2 min). We treated the extracts with alkaline phosphatase and exonuclease I (FastAP and ExoI, ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. We submitted extracts to Amplicon Express (Pullman, WA, USA) to determine forward and reverse DNA sequences using the same primers used for PCR amplification. Representative sequences of all 4 target loci for the gentotypes herein are available in Genbank: strain 393 (KU986495, KU986500, KU986503, KU986506, representing IGS, 16S, *rpoB*, and *gyrB*), strain 404 (KU986496, KU986501, KU986504, KU986507), strain 415 (KU986494, KU986499, KU986502, KU986505), strain 402 (KU986493, KU986498, representing IGS and 16S), and strain 419 (KU986492, KU986497).

Statistical Analysis

We used a Kaplan–Meier staggered entry estimator and a Cox proportional hazard model to analyze survival of females and lambs. We fit trend lines to 7-day moving averages of clinical scores using a lowess smoothing factor derived from locally weighted moving mean scores spanning 25% of the full dataset (2–3 weeks). We used a Fisher's exact test to analyze prevalence of pneumonia agents before, during, and after the outbreak and to compare agents present in adult and lamb mortalities. We used a Kruskal–Wallis rank-sum test to determine whether neutralizing titers to PI-3 differed before, during, and after the outbreak. We used a 1-way analysis of variance (ANOVA) and Tukey contrasts to test for differences in serologic antibody titers to *M. ovipneumoniae* over the course of the outbreak and a 2-sided *t*-test to compare pre-outbreak antibody titers to *M. ovipneumoniae* in animals that died and those that survived. We used a Wilcoxon rank sum test with continuity correction to analyze duration of clinical signs. We conducted analyses with package survival (Therneau 2015), and stats and base packages in R (R Core Team 2015).

RESULTS

Survival and Clinical Signs of Disease

In 2013, we observed all marked females with lambs, and median parturition date was 18 May (range = 5–20 May). In 2014, we observed 10 of 13 females with live lambs and median parturition date was 8 May (range = 27 Apr–17 May). One marked female and her lamb died, presumably of dystocia, on 16 May 2014 (e5 and L5, Fig. 1a), 1 marked female was observed with a dead 2-day old lamb (e10, Fig. 1a), and 1 unmarked female appeared to be pregnant but was never observed with a lamb (e22, Fig. 1a).

We observed clinical signs of pneumonia in lambs starting 1 June in 2013 and 26 May in 2014. Symptoms continued until the day the last lamb died, which was 30 June in 2013, and 2 July in 2014 (Fig. 1). Median lamb mortality date was 28 June in 2013 and 24 June in 2014, at a median age

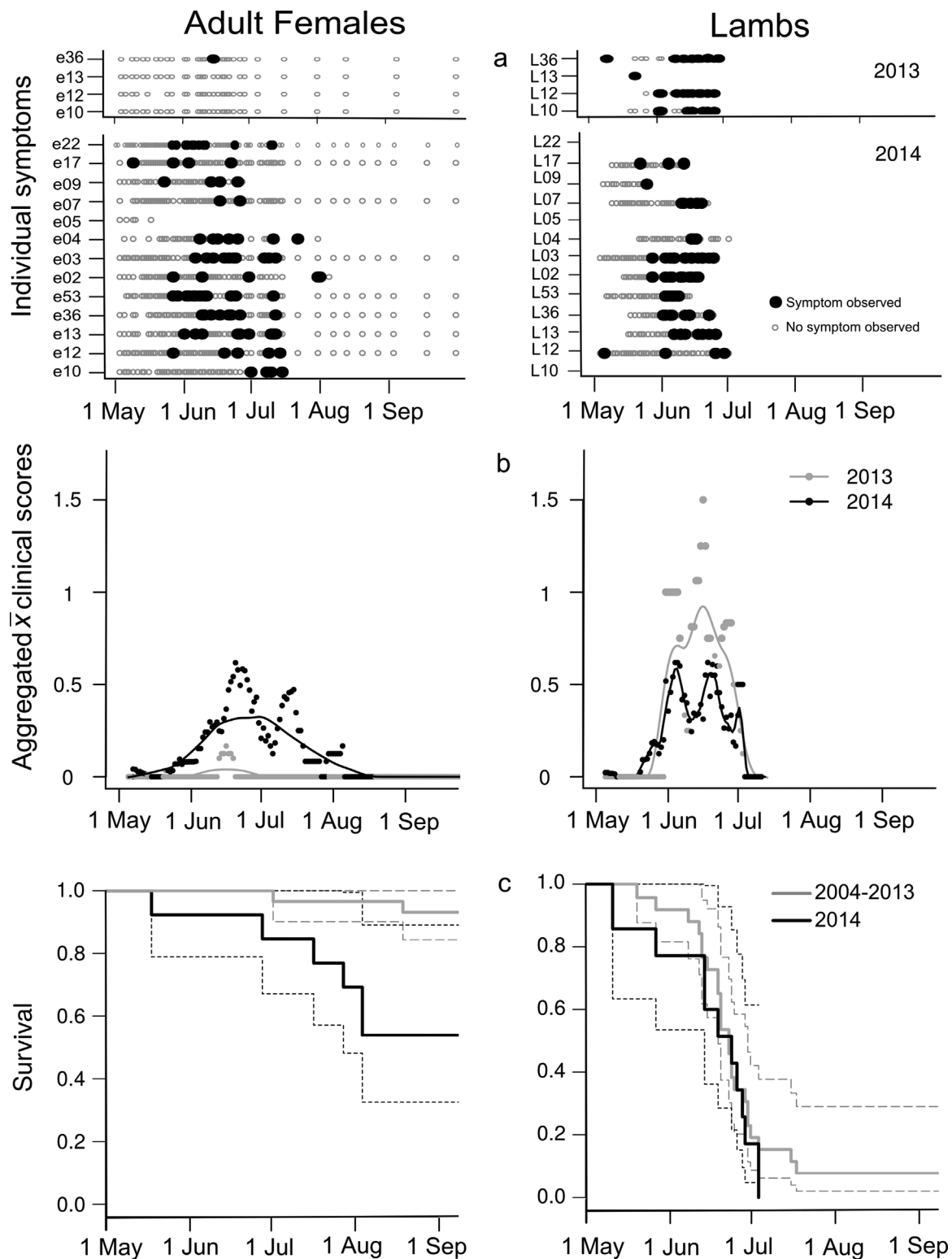


Figure 1. Clinical signs of pneumonia and survival of bighorn sheep in the Black Butte, Washington population during summers 2013 ($n = 4$ adult F and 4 lambs) and 2014 ($n = 13$ adult F and 10 lambs). (a) Time series of field observations of pneumonia symptoms in adult females (e) and their lambs (L). Observations ending prior to September indicate individual died. (b) Smoothed average daily clinical scores for adult females and lambs. (c) Adult female and lamb survival between May and October 2014 and survival between May and October during the 10 previous years, 2004–2013 (Kaplan–Meier curves and 90% CIs).

of 40 and 44 days, respectively. All lambs died both years and there were no differences in lamb survival curves between years ($\log \text{rank}_1 = 0.01$, $P = 0.92$) or between 2014 and the previous 10 years (Fig. 1c). We submitted 11 lambs

for necropsy (6 in 2013 and 5 in 2014). With the exception of 1 lamb that died at approximately 2 days of age in 2014 (L10, Fig. 1a), all lambs presented characteristic lesions of bighorn lamb respiratory disease, including moderate to

Table 2. Prevalence of *Mannheimia* spp. (Mh), *Bibersteinia trehalosi* (Bt), *Pasteurella multocida* (Pm), Pasteurellaceae leukotoxin encoding gene (*LktA*), and *Mycoplasma ovipneumoniae* (*Movi*) in asymptomatic bighorn sheep females (F) sampled before and after a pneumonia outbreak, symptomatic females during the outbreak, and in the lungs of pneumonic females that died in the Black Butte population and pneumonic lambs that died in Black Butte, (10) and adjacent populations (2), Washington and Oregon, USA.

Agent	F before (n = 11)	F during (symptomatic) (n = 2)	F pneumonia mortalities (n = 3)	Lamb pneumonia mortalities (n = 12)	F after (n = 8)
Mh	0.91	1.00	0.00	0.70	0.75
Bt	0.82	1.00	1.00	0.80	1.00
Pm	0.73	1.00	1.00	0.10	1.00
<i>LktA</i>	0.27	0.50	0.33	0.50	0.38
<i>Movi</i>	0.09	1.00	1.00	1.00	0.13

severe, subacute to chronic bronchopneumonia and otitis media.

Median age at first observation of clinical signs in lambs was 20 days (range: 7–35), median duration of clinical signs was 22 days (range: 1–59), and median age at death was 42 days (range: 22–66). Assuming that lambs were infected by 4 days of age and lung lesions were present by 10 days of age (Besser et al. 2008), we estimated a median latent period of 16 days (range: 3–31) between infection and first observation of disease symptoms in lambs and a median infection period of 38 days (range: 18–62) prior to death.

In adults, the only evidence of possible respiratory disease in 2013 was a single observation of coughing on 14 June, and all adults survived. In 2014, we observed clinical signs of pneumonia in adults starting in May. By June, all adults exhibited symptoms of pneumonia including severe prolonged coughing (Fig. 1a and b). Five adult females (38%) died between May and August 2014 (difference between summers, log rank₁ = 5.98, $P = 0.01$) and the hazard of an adult female dying in the summer of 2014 was 6.83 times higher (SE = 0.83, $P = 0.02$) than in any of the previous 10 summers (2004–2013, Fig. 1c). We did not observe pneumonia symptoms prior to the first adult death on 16 May 2014. Subsequent mortalities followed observation of clinical signs of pneumonia and occurred on 26 June, 18 July, 27 July, and 3 August (Fig. 1a). Median duration of clinical signs was 36 days (range = 18–81 days), which was longer than observed in lambs (median = 22 days, range = 1–59, $W = 136.5$, $P < 0.001$). Duration of clinical signs did not differ between adults that died and those that survived (Fig. 1a, Wilcoxon rank sum $W = 20$, $P = 1.0$). We submitted samples to WADDL from all (4 of 5) mortalities where sufficient tissues were available for diagnosis. No gross or histological evidence of respiratory disease was found in the female that died on 16 May, although evaluation of tissues at WADDL was limited because of autolysis. The 3 adult females submitted for necropsy between late June and early August were diagnosed with chronic, moderate to severe bronchopneumonia. The survivors appeared to make a full recovery with no evidence of ongoing disease.

Microbiology and Immune Responses

M. ovipneumoniae was the only pneumonia agent detected more frequently in the lungs of adults that died of pneumonia than in the upper respiratory tract of healthy

adults before and after the outbreak ($\chi^2 = 26.66$, $P < 0.001$; Table 2). Prevalence of other suspected pneumonia agents in the upper respiratory tract of symptomatic adults sampled during the outbreak was similar to that detected in the lungs of adults that died of pneumonia except that *Mannheimia* spp. were detected in the upper respiratory tract of live adults but not in the lungs of adults that died. We found no differences in prevalence of pneumonia agents present in upper respiratory tract samples collected before and after the outbreak ($\chi^2 < 2.6$, $P > 0.2$) or in the lungs of lambs that died in 2013 and 2014 ($\chi^2 < 0.39$, $P > 0.5$). Detection of *Mannheimia* spp. was more common in lamb mortalities than in adult mortalities ($\chi^2 = 4.55$, $P = 0.07$), and *P. multocida* was more frequently detected in the lungs of dead adults than lambs ($\chi^2 = 8.78$, $P = 0.01$; Table 2).

We detected serologic evidence of exposure to PI-3 (VN titer ≥ 4) and *M. ovipneumoniae* (ELISA inhibition $> 50\%$) in adults before, during, and after the 2014 pneumonia outbreak. We did not detect evidence of exposure to other respiratory viruses during the study. Average *M. ovipneumoniae* ELISA percent inhibition (%I) values prior to the outbreak (69%) increased significantly to 89%I and 82%I during and after the disease event, respectively ($F_{2,18} = 4.811$, $P = 0.02$; Fig. 2a). Individuals that died during the outbreak had higher *M. ovipneumoniae* ELISA % I values prior to the outbreak ($\bar{x} = 79\%$) than those that survived ($\bar{x} = 59\%$, $t_{7.5} = 3.02$, $P = 0.02$; Fig. 2b). Median PI-3 titers of 256 (log₂ 7.7) did not change significantly over the course of the outbreak ($\chi^2 = 4.55$, $P = 0.23$).

Strain Typing

We genotyped *M. ovipneumoniae* from all Heller Bar bighorn sheep where it was detected in 2013 ($n = 7$) and 2014 ($n = 11$). We found a single strain in 2013 based on identical DNA sequences of each of the 4 MLST loci, and that strain was also detected in the first lamb to die of pneumonia in 2014. All subsequent detections of *M. ovipneumoniae* differed from the 2013 strain at 3 MLST loci. The IGS sequences differed by 32 single nucleotide polymorphisms (SNP) and 3 base insertions or deletions (indels, 8.7% divergent), *rpoB* by 16 SNP (2.8% divergent) and *gyrB* by 16 SNP (4% divergent). The 16S sequences did not differ between strains.

We report strain differences by IGS sequences because we were unable to amplify *rpoB* and *gyrB* from DNA extracted

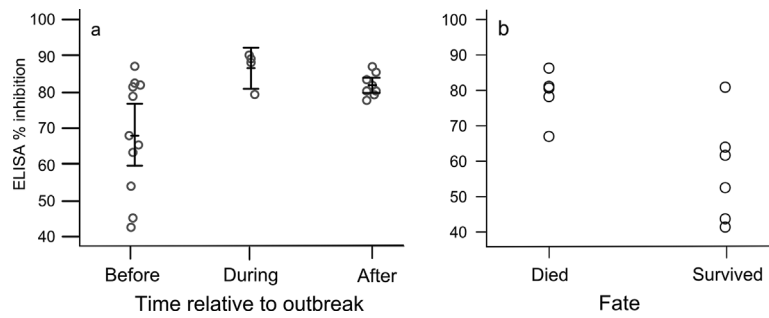


Figure 2. Serologic titers (competitive enzyme-linked immunosorbent assay [ELISA]) to *Mycoplasma ovipneumoniae* in bighorn sheep in Black Butte, Washington, February–October 2014. (a) Mean and 90% confidence intervals before, during, and after a pneumonia outbreak. (b) Antibody titer levels before the outbreak in adults that survived ($\bar{x}$ = 59%) and those that died ($\bar{x}$ = 79%).

from formalin-fixed, paraffin-embedded lung tissues, the only specimens available prior to 2006. Because each of these strains differed by indels in IGS, the strains are conveniently designated by their differing IGS lengths. The 2013 Black Butte strain, IGS 404, has been detected in this population since the 1995 pneumonia outbreak (Fig. 3b). The 2014 strain, IGS 393, had never previously been detected in this population or any other bighorn sheep population in Hells Canyon or elsewhere in the western

United States (among >700 other isolates that have been IGS typed).

In October 2014, we removed survivors from the Heller Bar female group in an attempt to prevent further spread of this strain. Nonetheless, 1 month after the removal, we detected the IGS 393 strain type in an adult male removed from the town of Asotin, Washington and we detected it again 4 months later in a 9-month-old lamb in the Shumaker female group, located between the Joseph and Heller Bar

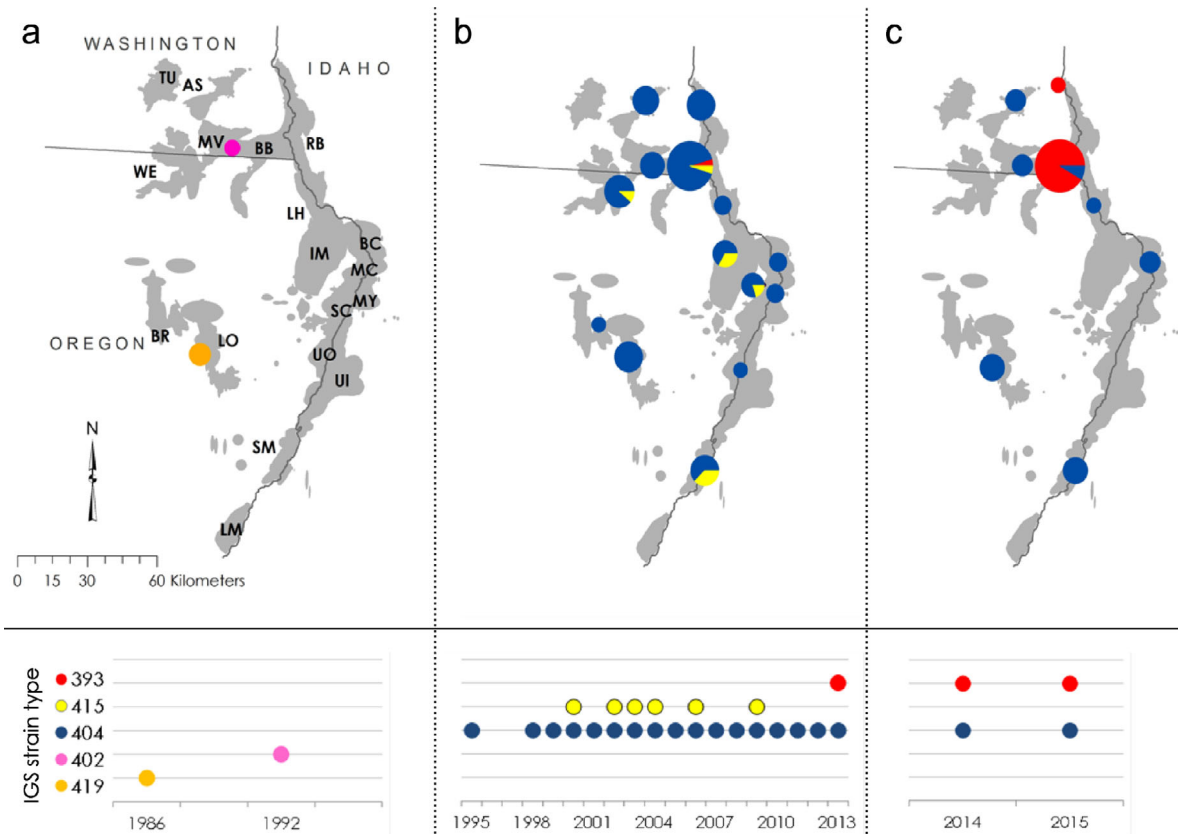


Figure 3. Spatiotemporal distribution of the 16S-23S intergenic spacer (IGS) genotypes of *Mycoplasma ovipneumoniae* in the Hells Canyon bighorn sheep metapopulation in Washington, Oregon, and Idaho, USA in (a) 1986 and 1992 (b) 1995–2013; and (c) 2014–2015. Each colored marker represents one strain type, pie charts display strain types within populations and are scaled by sample size. Pie charts containing >1 color indicate that 2 strain types were present in a population during that time interval but not necessarily detected in the same year. Gray shaded polygons denote bighorn sheep populations: AS = Asotin, BB = Black Butte, BC = Big Canyon, BR = Bear Creek, IM = Imnaha, LH = Lower Hells Canyon, LM = Lookout Mountain, MU = Muir, MV = Mountain View, MY = Myers Creek, RB = Redbird, SM = Sheep Mountain, TU = Tucannon, UO = Upper Hells Canyon, Oregon, UI = Upper Hells Canyon Idaho.

groups in the Black Butte population. Retrospective analysis revealed that this strain was present in the lungs of a male that died of pneumonia in the Joseph Creek group in December 2013, representing the index case of disease associated with the IGS 393 strain type. The IGS 393 strain has not been detected in any surrounding populations, which remain carriers of the original IGS 404 strain (Fig. 3c).

Two other strains were detected in specimens obtained during or after disease outbreaks in other Hells Canyon populations (Coggins 1988, Foreyt et al. 1990), and have not been detected since. These samples were from the Lostine population in 1986–1987 (IGS 419) and the Mountain View population in 1992 (IGS 402; Fig. 3a). A third strain (IGS 415) associated with a pneumonia outbreak in the Sheep Mountain population in 2000 was apparently replaced by the IGS 404 strain by 2006 based on samples typed from 2006 and 2015 in that population. The IGS 415 strain was subsequently infrequently detected in 4 other populations between 2003 and 2009 and has not been found since then (Fig. 3b).

Sequence divergence among the 3 recently detected strains where all 4 genes could be analyzed (i.e., IGS 393, 404, and 415) was between 9% and 10%. These strains were well dispersed across 20 genotypes of *M. ovipneumoniae* collected from 9 domestic sheep flocks in the western United States and Australia and 10 domestic goat flocks in the western United States and China. The IGS 404 and 415 types, first identified in the Black Butte and Sheep Mountain populations, respectively, were more closely related to the domestic sheep lineage, whereas the IGS 393 type detected in the Black Butte population in 2013 and 2014 clustered with the domestic goat clade (Fig. 4).

DISCUSSION

This is the first study to report on an all-age pneumonia outbreak in an intensively sampled population of free-ranging bighorn sheep with health, survival, and observational data collected before, during, and after the disease event. The collection and analysis of this information, employing recently developed molecular methods for pathogen detection and genotyping, allowed us to attribute severe disease in a bighorn sheep population with long-standing *M. ovipneumoniae* carriage to introduction of a novel strain of *M. ovipneumoniae*. This conclusion is supported by the detection of the never before recorded IGS 393 strain in the pneumonic lungs of adults that died, and in the upper respiratory tract of adults with clinical signs during the outbreak. We also observed a significant increase in antibody titers to *M. ovipneumoniae* during the outbreak denoting an active immune challenge and we documented that previous exposure and ongoing carriage of the *M. ovipneumoniae* IGS 404 strain was not protective against disease. To the contrary, lower survival of adults with higher serologic titers prior to the disease outbreak could reflect a harmful autoimmune reaction associated with antibody response to *M. ovipneumoniae* in bighorn sheep as has been suggested for domestic sheep (Niang et al. 1998a). Strain-specific immunity, as measured by serologic antibody

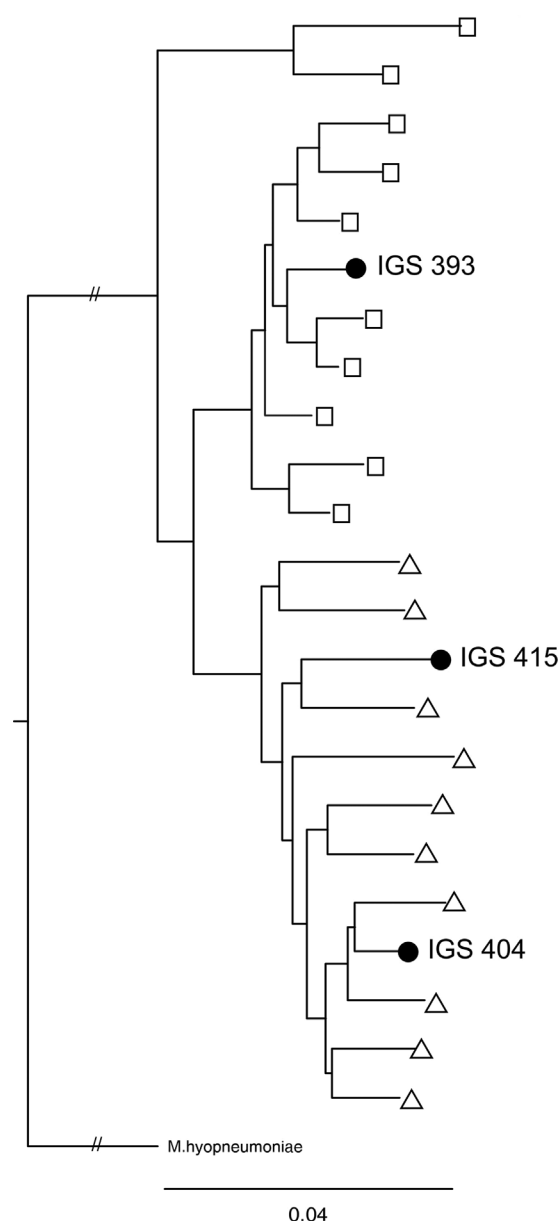


Figure 4. Phylogeny of 3 bighorn sheep strains of *Mycoplasma ovipneumoniae*, Hells Canyon, Washington, Oregon, and Idaho, USA, and randomly selected strains from domestic sheep (triangles) and domestic goats (squares), 1975–2015. Domestic sheep and goats appear to host divergent lineages of *M. ovipneumoniae* and the samples from bighorn sheep clustered with either the domestic sheep or domestic goat lineages. Neighbor-joining tree of concatenated partial 16S, 16S–23S intergenic spacer region (IGS), *rpoB*, and *gyrB* DNA sequences is rooted with homologous sequences from *M. hyopneumoniae*. Branch lengths are scaled by proportion non-identity (scale bar). Domestic sheep samples are from Washington, Oregon, Idaho, Nevada, Colorado, California, and an ATCC type strain from Australia. Domestic goat samples are from Washington, Idaho, California, and China (Yang et al. 2011, 2014).

inhibition of *M. ovipneumoniae*, was similarly reported by Alley et al. (1999) for domestic sheep.

Adult mortality associated with this strain introduction was within the range previously observed during pneumonia outbreaks in naïve animals in this metapopulation (28–42%; Cassirer et al. 2013). Lamb mortality followed an identical time course regardless of strain, consistent with a lack of

protective immunity in neonates. The timing of the onset of clinical signs following infection in lambs (latent period) was similar to that reported in experimental challenge of adults (Besser et al. 2014). However, disease progression in lambs was more rapid and severe than observed in free-ranging adults in this study or in experimental exposure of naïve adults in captivity (Besser et al. 2014).

Although pneumonia in bighorn sheep is a polymicrobial disease, pathogens other than *M. ovipneumoniae*, including *lktA* positive *Pasteurellaceae* and respiratory viruses, were either not detected or showed no association with disease. Whereas *M. ovipneumoniae* was present in all pneumonic adults and lambs, prevalence of *Pasteurellaceae* varied between age classes. This could be due to conditions associated with growth of an opportunistic pathogen or to other factors. Sample sizes were too small to draw broader inference. Anaerobic bacteria, not tested in this study, are a large component of the microbiome in pneumonic bighorn sheep lung tissue and may also play a larger role as secondary pathogens than previously suspected (Besser et al. 2008).

The novel strain of *M. ovipneumoniae* detected in this study differed from the resident strain by 52 independent genetic mutations on 4 loci. This unique strain was not a variant of a resident strain and had never before been detected in over 700 samples strain-typed from Hells Canyon and other bighorn sheep populations. Therefore, the most likely source of this Caprinae-specific pathogen was a domestic sheep or goat. Phylogenetic analysis indicated that this strain was most likely of domestic goat origin.

This strain introduction likely occurred from a domestic goat on or from private lands within bighorn sheep range despite substantial efforts by wildlife managers and nongovernmental organizations to prevent contact. Management strategies included distributing educational material to flock owners and the general public, purchasing and removing a domestic sheep flock, and removing individual bighorn or domestic sheep and goats when they were at risk of contact. Our retrospective analysis showed that unique strains of *M. ovipneumoniae* were associated with 4 other epidemiologically unrelated all-age pneumonia outbreaks in Hells Canyon, as would be expected from similar spillover events. Two of these historical strains (IGS 402 and 419) apparently remained localized, one spread and subsequently disappeared (IGS 415), whereas the fourth (IGS 404) has persisted and proliferated over a span of ≥ 20 years. It is not clear why some strains of *M. ovipneumoniae* persist and others apparently do not. The IGS 404 strain may have driven fade-out of other extant strains when it spread, as introduction of the IGS 393 strain did in this study. Strain replacement might occur when the carrier host immune response is cross-reactive but protection is strain-specific. Under these conditions a new strain could have a competitive advantage and exclude the original strain.

Although evidence suggests that this epizootic was caused by introduction of a novel *M. ovipneumoniae* strain, it is also possible that pneumonia outbreaks could be precipitated in carrier bighorn sheep populations if appropriate mutations occur in strains to which resistance has previously been

acquired. Mutations in key virulence genes or in genes coding for the *M. ovipneumoniae* capsule, which likely plays a role in adherence to host cells and in evading antibody recognition (Niang et al. 1998b, Razin et al. 1998), could cause disease and are unlikely to be detected by our strain-typing method. Another plausible mechanism of pneumonia resurgence would be reintroduction of the same strain of *M. ovipneumoniae* into a population following either pathogen fade-out and waning immunity (Sydenstricker et al. 2005) or recruitment of unexposed susceptible individuals. Finally, other factors may play a role in triggering outbreaks such as pathogen dose, host contact patterns, immunocompetence, and invasion of secondary pathogens. Identifying the conditions most frequently associated with pneumonia outbreaks in previously exposed populations and a wider investigation of the genetic diversity and host-specificity of *M. ovipneumoniae* strains would provide valuable insights into the adaptive immune response in bighorn sheep and the ecology of this disease.

MANAGEMENT IMPLICATIONS

Lack of cross-strain immunity to *M. ovipneumoniae* could be one explanation for the regular occurrence of pneumonia epizootics in bighorn sheep populations over a century after initial contact with domestic sheep. Single strain infection in bighorn sheep populations contrasts with *M. ovipneumoniae* carriage in domestic sheep where numerous strains typically coexist within a flock (Alley et al. 1999, Harvey et al. 2007). In the absence of cross-strain immunity, these flocks may serve as a constant source of novel strains capable of causing disease in bighorn sheep. Although vaccination could potentially reduce pathogen burden or prevalence within bighorn sheep populations, it is not clear that a vaccine would protect bighorn sheep from severe disease if exposed to new strains. Our results instead support preventing spillover as a primary strategy for managing disease in bighorn sheep. This could be accomplished by maintaining separation between bighorn sheep and domestic sheep and goats, by clearing *M. ovipneumoniae* infection from domestic hosts, and by exercising caution to avoid mixing *M. ovipneumoniae* strains among bighorn sheep populations during translocations. The management strategies implemented near the bighorn sheep in this study were apparently unsuccessful in preventing transmission, underscoring the difficulty of maintaining separation. New approaches, more active cooperation by the public, and greater vigilance on the part of resource managers may be key to preventing pneumonia outbreaks in bighorn sheep.

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RISK FACTORS AND PRODUCTIVITY LOSSES ASSOCIATED WITH *Mycoplasma ovipneumoniae* INFECTION IN UNITED STATES DOMESTIC SHEEP OPERATIONS

A PREPRINT

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ABSTRACT

Association of *Mycoplasma ovipneumoniae* with pneumonia in domestic small ruminants has been described in Europe, Asia, and New Zealand but has received less attention in the United States. In 2011, the US Department of Agriculture’s National Animal Health Monitoring Survey detected *M. ovipneumoniae* shedding in 88% of 453 domestic sheep operations tested in 22 states that accounted for 85.5% of US ewe inventory in 2001. We evaluated factors associated with *M. ovipneumoniae* infection presence and prevalence, and we compared health, lamb production, and ewe losses in infected and uninfected operations. *M. ovipneumoniae* detection was more common in larger operations than in smaller operations. Both likelihood of detection (at the operation level) and within-operation prevalence were higher in operations with more open management practices than in operations with more closed management practices. *M. ovipneumoniae*-positive operations showed significantly lower lambing rates and lower rates of lamb survival to weaning after accounting for differences in operation size and management practice. While its effect on any single rate was not particularly large, in aggregate we estimated that *M. ovipneumoniae* presence was associated with an approximately 4.3% reduction in annual lamb production.

Keywords sheep, respiratory disease · *Ovis aries* · *Mycoplasma ovipneumoniae* · productivity · prevalence

1 Introduction

Mycoplasma ovipneumoniae is a recognized pathogen of Caprinae [1, 2, 3], and has been associated with transmissible respiratory disease in domestic sheep in experimental settings [3]. Like other respiratory mycoplasmas, *M. ovipneumoniae* initially colonizes the respiratory tract, where it impedes movement of the ciliary escalator [4, 5]. This allows a variety of normally commensal upper respiratory, rumen, and inhaled flora to invade the lungs, where they can cause chronic, polymicrobial pneumonia [6, 4, 7].

Veterinary texts describe *M. ovipneumoniae*’s impact on animal health and operation productivity with characterizations ranging from “mild” (in Smith’s Large Animal Internal Medicine[8]) to “proliferative” (in Radostits’s Veterinary Medicine [9]). The diversity of descriptors leaves ambiguity about how veterinarians and operators should view *M. ovipneumoniae* infection in terms of production loss. Respiratory disease is a serious problem for domestic sheep production in the United States, and is the fifth-highest source of lamb loss, following weather, lambing problems, predation, or unknown causes, and on a par with gastrointestinal problems [10, 11]. Additionally, there are only limited and local data on *M. ovipneumoniae* presence in United States domestic sheep operations [6, 12, 13, ?]. A better understanding of the risk factors associated with *M. ovipneumoniae* infections, and the burden these infections place on domestic sheep production would help producers determine how to prioritize its management.

Here, we investigate whether *M. ovipneumoniae* is associated with reduced productivity in United States domestic sheep operations. We postulate that *M. ovipneumoniae* may be an under-recognized pathogen of domestic sheep in the United States for two reasons. First, disease burdens associated with other *Mycoplasma* infections of livestock are well-documented (e.g., [14]). This is true, for example, of *Mycoplasma mycoides*, the causal agent of contagious bovine pleuropneumonia, and *Mycoplasma capricolum*, the causal agent of contagious caprine pleuropneumonia, as well as the disease burden associated with *M. ovipneumoniae*’s close relative *Mycoplasma hyopneumoniae*, a pathogen of pigs (e.g., [15, 16]). Second, *M. ovipneumoniae* is thought to cause chronic disease and generate important mortality burdens in domestic sheep in other parts of the world [3, 2]; as well as in other caprine hosts, including domestic goats [17, 18]. However, despite burdens associated with *M. ovipneumoniae*’s close relatives in domestic animals, and with *M. ovipneumoniae* itself in other caprinae hosts, little work has explored the burden of *M. ovipneumoniae* on domestic sheep production within the United States.

We describe the distribution of *M. ovipneumoniae* in domestic sheep operations throughout the United States using data acquired through the U. S. Department of Agriculture National Animal Health Monitoring System. Our inquiry is built around two questions. First, can we identify specific management practices associated with *M. ovipneumoniae* infection that would allow us to predict — and potentially reduce — infection status and prevalence? Second, what are the consequences of *M. ovipneumoniae* infection on specific production and aggregate productivity for United States domestic sheep operations?

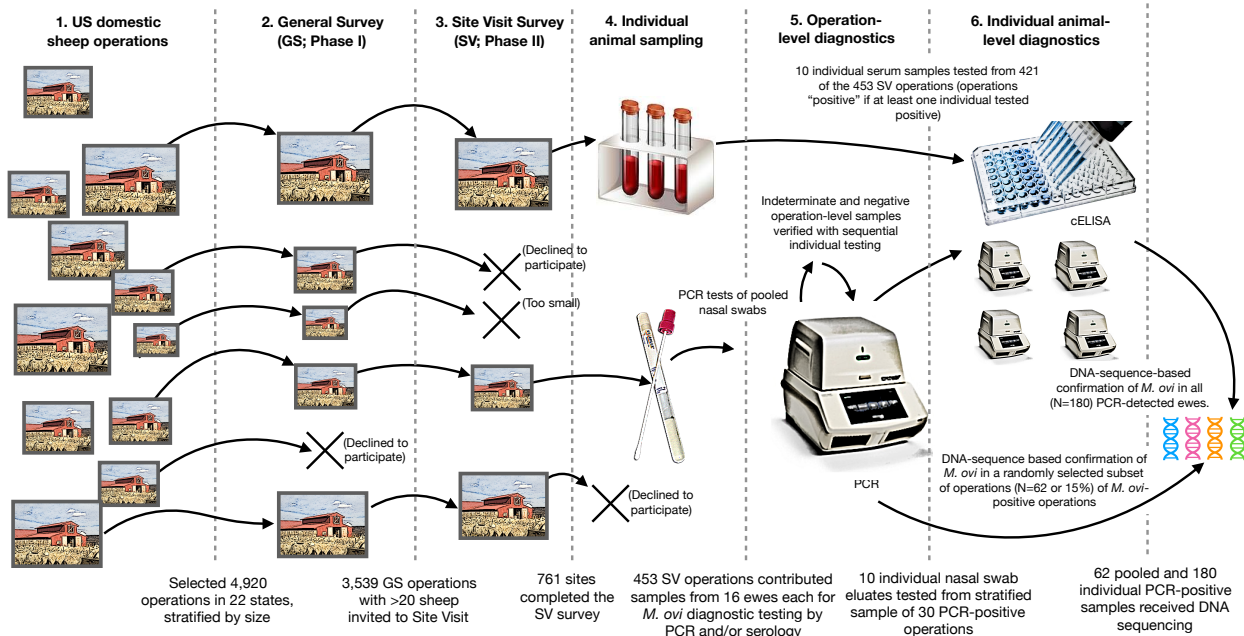


Figure 1: **Sampling and data development.** Operations were identified prior to Phase I of the NAHMS Sheep-2011 survey, and a size-stratified sample of 4,920 operations were invited to participate in the General Survey Phase I. A subset of General Survey Phase I operations elected to participate in the Site Visit (Phase II) portion. Of that subset, a smaller group (761) completed the survey. Of these, 453 participated in the *Mycoplasma ovipneumoniae* portion of the study. Sixteen animals at each of those 453 operations contributed nasal swabs and venous blood samples. Those samples were developed individually, and then pooled and tested by PCR and cELISA. When an operation's pooled PCR result was indeterminate or negative, individual samples from that operation were tested sequentially until an animal tested PCR-positive, or all tests were queried. Ten animals each from a structured subsample of 30 operations PCR-positive on the pooled test were then tested at the individual level to establish operation-level prevalence estimates. A random subset of 62 pooled operation samples, and all 180 individual animal samples that tested PCR-positive in the prevalence estimation were then sequence-confirmed as being *M. ovipneumoniae*-positive.

2 Materials and methods

2.1 Data collection and laboratory analyses

2.1.1 USDA National Animal Health Monitoring Survey (NAHMS) Data

Data were collected through the USDA-APHIS-VS National Animal Health Monitoring System (NAHMS) on domestic sheep production conducted in 2011 (Figure 1). Like most NAHMS studies [19, 20], the survey consisted of two stages. First, premises were selected from a sampling frame of all domestic sheep operations (those with one or more sheep on hand on January 1, 2010; [21]) in 22 states accounting for 85.5% of United States domestic sheep production [21]. A sample of 4,920 operations, stratified by state and operation size, was drawn from this frame. Participants completed a General Survey ("GS"; Phase I) questionnaire between January 1st and February 11th, 2011 (USDA 2012), which covered a range of issues relevant to domestic sheep production. As in other NAHMS surveys [19, 20], the 3,539 Phase I operations with 20 or more ewes (71.9% of all GS operations) were then invited to participate in a second site visit ("SV"; Phase II) phase of the study, which included sample collection. One thousand, two hundred and forty-one (35.1%) of the GS operations with 20 or more animals agreed to participate in the site visit portion of the survey ([10] pg. 145), of which 761 (61.3%) actually contributed complete information ([10] pg. 150; Figure 1). Of those, 453 operations (59.5%) also contributed the biological samples forming the basis for the analysis presented here.

2.1.2 Collecting samples from domestic sheep operations

At each participating operation, the Veterinary Medicine Officer conducting the Phase II site visit obtained nasal swab samples from 16 ewes (Figure 1). This sample size was chosen by NAHMS to balance pre-specified precision estimates

for detecting *M. ovipneumoniae* presence (not prevalence) given estimated flock and animal-level prevalence against necessary budget constraints. A single swab (BBL CultureSwab EZ 220144) was used to swipe both nares from each sampled animal, and was then placed in mycoplasma transport media. All 16 nasal swabs from each operation were shipped to the Washington Animal Disease Diagnostic Laboratory (WADDL). Blood samples (10 ml) were obtained by jugular venipuncture from the same 16 sampled ewes, collected into red top (clot) tubes, and shipped to the National Veterinary Services Laboratory, where serum was extracted and aliquoted. One set of serum aliquots was then shipped to WADDL for testing.

2.1.3 Detecting *Mycoplasma ovipneumoniae* by PCR

At WADDL, nasal swabs were placed in mycoplasma broth media (R102, Mycoplasma Broth, Hardy Labs, Santa Maria CA) and incubated at 35C for 72-96 hrs. Following incubation, aliquots of post-enrichment broths from the 16 ewes were pooled into a single operation-level sample. DNA was extracted from the pooled sample (MagMAX, ThermoFisher Scientific, Waltham MA) and tested using PCR for *M. ovipneumoniae* [22]. Operations with pooled samples producing cycle threshold (CT) values of 34 or less were classified as “infected”. If pooled samples produced CT values >34, individual nasal swab enrichment tube samples were tested. Operations in which all pooled and individual nasal swab enrichment tube samples produced CT values >34 were classified as “not infected” (Figure 1).

To determine operation-level prevalence of *M. ovipneumoniae* carriage, 30 operations were randomly selected for realtime PCR testing of mycoplasma enrichment broths from ten ewes each. After initial analysis of the specimens from this project in 2011, the realtime PCR method for detection of *M. ovipneumoniae* was modified to eliminate false positive reactions associated with the presence of an unidentified Mycoplasma-like organism. To ensure consistency in diagnostic methods, this modified realtime PCR method was used to retest all available DNA extracts in which *M. ovipneumoniae* had originally been detected. The modified version of the WADDL realtime PCR included 1) use of a new primer (226Fnew 5'-GGGGTGCGCAACATTAGTTAGTTGGTAG-3') to replace the previously described forward primer [22], 2) modified master mix to include bovine serum albumin (final concentration, 400 ng/ml), and 3) modified thermocycling conditions including Stage 1: 50C hold for 2 minutes followed by 95C hold for 10 minutes; optics off; Stage 2: 40 repeat cycles of denaturation (95 C, 15 seconds, optics off) and annealing/extension (66 C, 60 seconds, optics on). The results of the modified realtime PCR were interpreted as follows: ‘detected’ if the cycle threshold score (CT) was 36 or lower, ‘indeterminate’ for CTs between 36 and 40, and ‘not detected’ for a CT of 40.

Verifying accuracy of *M. ovipneumoniae* detection

To ensure consistency of *M. ovipneumoniae* detection, the modified WADDL realtime PCR described previously was also used to re-test specimens from all available operations classified as *M. ovipneumoniae*-positive based on the WADDL 2011 test method. Of the 401 operations classified as *M. ovipneumoniae*-positive based on the 2011 test, DNA specimens were available for re-testing from 372 operations.

Both the original and the modified methods were also used for determining sequences of multiple loci for comparison with reference strain data [23]. In addition, for some samples a different partial 16S rDNA target sequence was used, amplified by the primers 226Fnew and 1025Rnew (5'-ATCTCGTTAGCCTCCGCTATATCT-3') with the thermocycling conditions Stage 1: 95C hold for 5 minutes; Stage 2: 45 cycles of denaturation (95 C, 30 seconds), annealing (66.5C, 30 seconds) and extension (72 C, 60 seconds); and State 3: 72C hold for 10 minutes. Sequence confirmation was sought from DNA extracts from the pooled operation-level enrichment broths from a randomly selected subset of 62 *M. ovipneumoniae*-infected operations. Sequences obtained were used to search GenBank using the Basic Local Alignment Search Tool (BLAST) to detect the most similar database entries. In addition, DNA sequence confirmation was applied to DNA extracts (N=180), which included all individual ewe samples from the 30 randomly selected operations in which *M. ovipneumoniae* was detected by the modified WADDL realtime PCR method. DNA sequences resulting from these efforts have been deposited in GenBank (MH042304-MH042516; MH045511-MH045514; MH087248-MH087420; MH107389-MH107763).

2.2 Data analysis

2.2.1 Response variables

The analysis was built around two separate blocks of response variables. In the first block, *M. ovipneumoniae* was treated as a response, and we analyzed *M. ovipneumoniae* presence (0 or 1, based on the pooled operation-level samples) and, given a positive operation, prevalence (proportion 10 ewe samples that tested PCR-positive, for the subset of operations receiving follow-up testing) as a function of a set of potential risk factors. In the second block, *M. ovipneumoniae* was treated as a predictor, and we explored how ewe survival and productivity was measured by number of pregnant ewes lambing at full term, number of lambs born per ewes bred, number of birthed lambs surviving to weaning, lamb weight

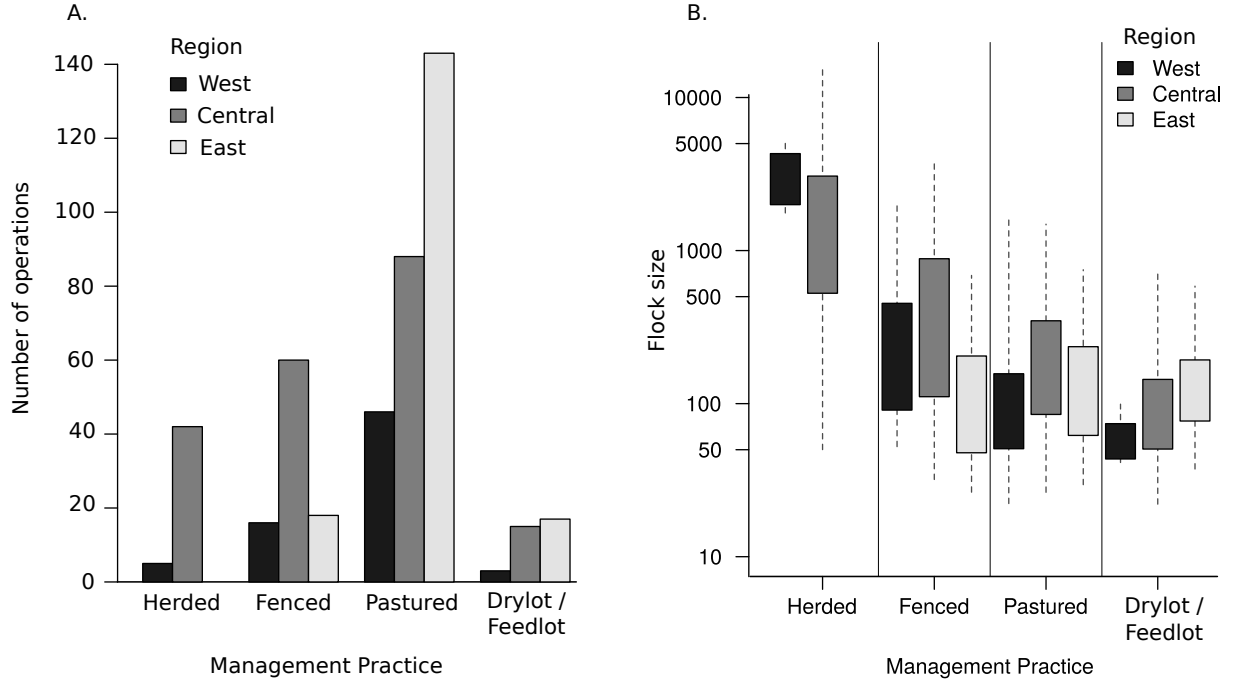


Figure 2: **Sheep management styles.** (a) Distribution of region-level flock management practices across 453 operations that contributed biological samples during the Site Visit portion of the NAHMS Sheep-2011 survey. (b) Logged herd size across four different herd management practices, and split out by region. Boxes capture the middle 50% of operations in each group; lines extend to the 2.5th and 97.5th quantile in each group.

at weaning, and number of sheep dying from respiratory disease — varied according to *M. ovipneumoniae* presence and prevalence.

2.2.2 Potential predictors of *M. ovipneumoniae* infection

We considered five risk factors that we hypothesized could influence a flock’s likelihood of being infected with *M. ovipneumoniae*, and might affect *M. ovipneumoniae* prevalence within infected flocks. The risk factors were 1) operation size; 2) primary management type; 3) an index for operation biosecurity (ranging -1 to 10); 4) an index for overall disease burden (ranging 0 to 11); and 5) an index for operation-level antibiotic use practices (ranging 0 to 5). Primary management types were “fenced” operations where animals were contained in any fenced area not specifically cultivated to raise forage or browse; “herded” operations where animals were maintained on any unfenced acreage; or “pastured” operations where animals grazed fenced areas specifically cultivated to raise forage or browse [21]. Data from 35 dry lot and feedlot operations that contributed biological samples through the Site Visit portion of the study but that did not allow grazing were excluded due to limited occurrence in our dataset. The three index variables were created by aggregating each operation’s responses across a series of General Survey and Site Visit questions. Contributing survey questions are listed in Table S1, and univariate distributions of each index are shown along the main diagonal of Figure S1.

Regions varied substantially in management type and operation size (Figure 2), which confounded management type and region. In particular, operations that pastured their animals were disproportionately located in the East region, whereas operations that used fenced or herded management practices were disproportionately located in the Central region (Figure 2). Since there is no clear mechanism for regional difference in *M. ovipneumoniae* persistence outside the host (*M. ovipneumoniae*’s environmental persistence is thought to be uniformly poor due to its lack of a cell wall, [24], and in an effort to focus our analyses and protect participant privacy, we chose to omit Region throughout the remainder of this investigation.

We first evaluated univariate relationships between each risk factor and *M. ovipneumoniae* presence across all fenced, pastured, and herded operations contributing biological samples to NAHMS Sheep 2011 Phase II using Wilcoxon rank-sum tests, and then we fit a logistic regression model in which operations were assigned a one if they were *M.*

ovipneumoniae-positive, and a zero otherwise. All covariates were included as additive effects, since a subset of models examining covariate interactions showed limited, or no improvement in model performance on the basis of AIC (all models with interactions were > 2 AIC points above model with only additive terms). Covariates were not substantially collinear with one another (absolute value of all correlations < 0.4 throughout; see Figure S1), so we included them all in subsequent models. An identical model with a binomial residual structure with $n = 10$ (number of animals individually tested by PCR in each operation) was used to characterize associations between the same set of risk factors and *M. ovipneumoniae* prevalence on the subset of 62 size-stratified operations subject to individual-level testing (see Detecting *Mycoplasma ovipneumoniae* by PCR).

2.2.3 Associations between vital rates and *M. ovipneumoniae* infection status

We examined the association of operation-level *M. ovipneumoniae* presence (zero for no *M. ovipneumoniae* detection) and prevalence in positive operations (number of individuals testing positive out of 10) with seven response variables linked to productivity and animal health. Response variables were full-term births among pregnant ewes, number of lambs born per bred ewe, lambs born that survived to weaning, average lamb weight at weaning, number of ewes culled, and number of ewes and lambs that died from respiratory disease. We built one set of models in which *M. ovipneumoniae* presence or absence was a predictor (fit to all operations with complete biological samples), and a second set in which *M. ovipneumoniae* prevalence was a predictor (fit to prevalence data from the stratified set of 62 operations where individual-level prevalences were established). All models also contained management type and operation size covariates to account for vital rate differences among production styles. We checked for correlations among transformed response variables, and they were negligible following transformation (Figure S4).

The proportion of pregnant ewes that deliver lambs at full term was treated as a binomial variable. The number of lambs born per bred ewe was treated as Poisson, with total number of lambs born. Lamb survival to weaning was treated as a binomial response variable. Lamb weight at weaning was modeled as Gaussian with an identity link; that model also incorporated average age at weaning, and an indicator variable of whether lambs were fed high-energy diets. We also examined the number of ewe, pre-weaning lamb, and weaned lamb deaths due to respiratory disease. These were treated as negative binomial variables to account for over-dispersion in the raw counts (many operations had no respiratory deaths in any age group). The negative binomial models also included total number of sheep on the operation as offset terms.

Additional descriptions of response variables, formal statements of all models, and specific model fits, are included in the Supplementary Materials.

2.2.4 Statistical analyses

All statistical analyses were conducted in R [25]. Linear and most of the generalized linear models were fit using the `bayesglm` function in the `arm` package [26] to circumvent any issues associated with complete separation – the situation in which a covariate completely splits a response, such that all positive (or negative) responses occur under a single level of the covariate. This might occur, for instance, if all operations of a particular management type tested PCR-positive for *M. ovipneumoniae*. `bayesglm` works as a simple augmentation of the usual iteratively reweighted least squares that is classically used to fit generalized linear models by first incorporating a small data augmentation to adjust for complete separation, and then running a step of iteratively weighted least squares and a step of expectation-maximization algorithm to estimate coefficient variances [27]. While the inference approach is fundamentally Bayesian in that it directly incorporates priors, it is structured to analogize cleanly with iteratively reweighted least squares; thus our inferences are presented as a hybrid of Bayesian and frequentist norms. Student's T-distributions with 2.5 degrees of freedom (or 10 degrees of freedom in the case of the intercept) for all coefficients with expectation-maximization updates were used as priors for all model coefficients.

To model over-dispersed count variables (e.g., count variables for which there are more zeroes than expected), negative binomial regression models were fit using the `bayes_glm` function in the `rstanarm` package [28]. Negative binomial models are typically chosen as alternative models for over-dispersed data because they naturally account for the extra-Poisson variability via an additional variance parameter [29, 30]. The `bayes_glm` function fits Bayesian generalized linear regression models in the Stan programming language [31], which uses adaptive Hamiltonian Monte Carlo sampling to simulate draws from the posterior distribution. These are fully Bayesian models, unlike those fit by the `bayesglm` function and thus do not generate p-values. Instead, posterior credible intervals are used to make inference about predictor variables of interest. Again, Student's T-distributions with 2.5 degrees of freedom (or 10 degrees of freedom in the case of the intercept) was used as the prior distributions for all model coefficients.

During a preliminary investigation, we relied on AIC-based model selection to identify best-performing models, and considered models with all possible combinations of covariates in the model suites. This exploration indicated that

models including additive effects of operation size and management nearly always performed best, and we used that covariate structure as the basis for all models presented here. We report McFadden's pseudo R-squared values as a coarse metric of performance on logistic regression model fits. Though widely used, pseudo R-squared values are not identical to conventional R-squared values available through ordinary least squares. Pseudo R-squared values are often lower than conventional R-squared values, and in general, values of 0.2 - 0.4 are considered "excellent fits" [32].

3 Results

3.1 Data overview and structure of sampled operations

A total of 453 operations voluntarily contributed biological samples for *M. ovipneumoniae* testing as part of the Sheep 2011 study. Ninety-two percent (418) of those operations were under fenced, pastured, or herded management practices, and of those 418, 10 contributed swabs but not serum samples. A cross-tabulation of operations where *M. ovipneumoniae* was detected via PCR, and where antibodies to *M. ovipneumoniae* were detected via cELISA, is shown in Table S2, along with a full description of cELISA methods.

Operation size and production type varied by region. Herding occurred in the Central and Western regions of United States, but not in the East. Operations using herded management systems were on average much larger (median = 1,750 animals; 95% of operations between 50 and 15,200 animals) than operations using pastured (median = 128 animals; 95% of operations between 26 and 1,444 animals) or fenced (median = 195 animals; 95% of operations between 31 and 2,867 animals) management. Although only 3.1% of operations used herded or open-range management, those operations accounted for 26.1% of ewes on operations with 20 or more animals (USDA-APHIS 2012). A comparison of the NAHMS Site Visit sites with data from all operations surveyed during the Sheep 2011 NAHMS General Survey suggested that Site Visit sites provided a reasonable cross-section of production sizes and management strategies used across all surveyed sites.

3.2 Reliability of diagnostic tests

M. ovipneumoniae was detected by the modified realtime PCR method in 369 of the 372 available specimens. Of the three discrepant samples, one produced 'indeterminate' results by the modified realtime PCR, while *M. ovipneumoniae* was not detected in the other two specimens by either the original or the modified realtime PCRs, suggesting that the DNA template had degraded during storage. Overall, the modified realtime PCR method detected *M. ovipneumoniae* in 369 of 370 (99.7%) of the available, template-intact specimens. We also used DNA sequencing to evaluate the accuracy of the realtime PCR methods using DNA extracts obtained from 1) 62 (15%) randomly selected operations classified as *M. ovipneumoniae*-infected, and 2) all enrichment broth specimens (N=180) in which *M. ovipneumoniae* was detected while estimating individual animal prevalence. All 62 randomly selected, pooled (operation-level) enrichment broths and all 180 individual ewe enrichment broths (composed of all samples in which *M. ovipneumoniae* was detected after 10 samples each were tested from 30 randomly selected operations) produced DNA sequences highly similar to *M. ovipneumoniae* GenBank entries. Together, these findings provide strong support for the accuracy of identification of *M. ovipneumoniae*-infected operations and individuals, and therefore, all results found to be positive by the original WADDL PCR test are defined as positive for these analyses. False negative misclassification of operations is another potential problem, and the limited numbers of ewes sampled per operation (N=16) predicts that there is a 5% or larger risk of false-negative misclassification of operations with an individual animal prevalence of 0.17 or lower (binary distribution). This risk of false negative classification may be the explanation for some or all of the 36 operations classified as *M. ovipneumoniae*-negative by PCR, but in which *M. ovipneumoniae* cELISA-reactive ewes were detected (Table S2).

3.3 *M. ovipneumoniae* presence and prevalence in surveyed operations

Of the operations contributing biological samples, 88.5% (401/453; 95% binomial confidence interval [85.2%, 91.3%]) tested PCR-positive for *M. ovipneumoniae*, and 85.3% (348/408; 95% confidence interval [81.5%, 88.6%]) showed signs of exposure to *M. ovipneumoniae* under the cELISA antibody assessment. *M. ovipneumoniae* prevalence varied substantially among the 62 size-stratified operations selected for individual-level testing (Figure S2). The median prevalence among those operations was 60%, though that figure should not be regarded as widely representative, given its basis on a stratified sample intended to cover a range of covariate values.

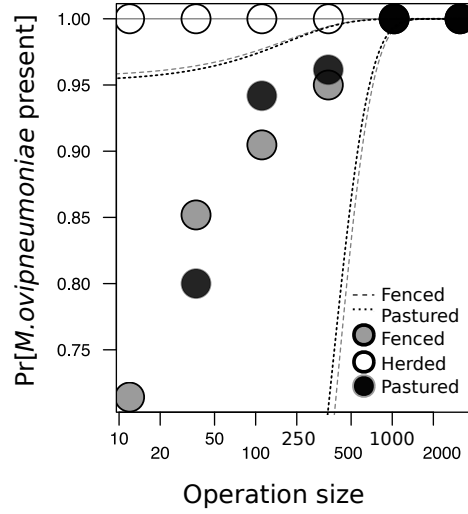


Figure 3: *M. ovipneumoniae* risk factors. Points show empirical proportions of operations containing PCR-detectable *Mycoplasma ovipneumoniae* infection, grouped by operation size and management type. Shaded regions show 95% credible intervals for the probability of *M. ovipneumoniae* detection in an operation, in a model that also accounted for operation size and management type. The dotted interval is for Pastured operations; the dashed interval is for Fenced (intervals overlap extensively). No interval could be calculated for operations with herded management, since all herded operations in this study showed active *M. ovipneumoniae* infections.

Table 1: USDA NAHMS Sheep 2011 survey coefficient estimates from a model of *M. ovipneumoniae* presence as a function of operation size, management type (baseline = Fenced), contact score, disease burden score, and antibiotic use score. This model was fit using the 408 operations that contributed biological samples, and where management was herded, pastured, or fenced.

Variable	Coefficient estimate	Standard error	Test statistic (p-value)
(Intercept)	0.754	0.406	1.855 (0.064)
management = Herded	1.768	1.469	1.203 (0.229)
management = Pastured	0.008	0.549	-0.515 (0.607)
operation size	0.005	0.002	2.819 (0.005)
contact score	0.072	0.062	1.722 (0.085)
disease burden score	-0.004	0.120	-0.029 (0.977)
antibiotic use score	0.379	0.295	1.281 (0.200)

3.4 *M. ovipneumoniae*-associated risk factors

All 47 herded operations tested PCR-positive for *M. ovipneumoniae*, as did 237 of 277 (86%) pastured operations, and 83 of 94 (88%) fenced operations. The median operation size of *M. ovipneumoniae*-positive operations was significantly larger (median size = 171 ewes) than negative operations (median size = 70 ewes, Wilcoxon p-value < 0.0001; Figure 3).

Biosecurity risk scores were marginally significantly higher in operations testing PCR-positive for *M. ovipneumoniae* than in operations testing negative ($W = 1503.5$, p-value = 0.069). There was evidence that PCR-positive operations were also subject to higher overall disease burden than negative operations (Wilcoxon rank-sum test $W = 7708.5$, p-value = 0.013). We found no significant association between antibiotic use and *M. ovipneumoniae* presence (Wilcoxon rank-sum test $W = 307.5$, p-value = 0.599).

In a logistic regression of *M. ovipneumoniae* presence as a function of the five postulated risk factors, only operation size and contact score retained their significance, and contact score only marginally so (Table 1).

The best explanatory model of *M. ovipneumoniae* prevalence within an operation for the 62 PCR-positive, individually sampled operations included only a single predictor, management type (AIC = 27.5). Models that also included

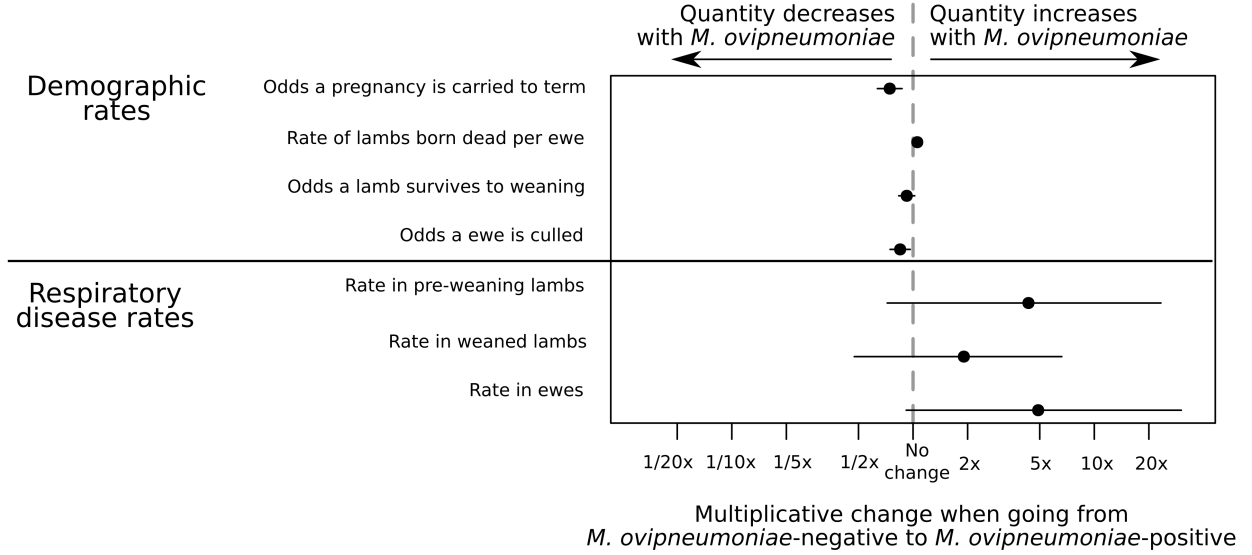


Figure 4: **Associations between *M. ovipneumoniae* presence and production rates.** Estimated changes in vital rates and rates of reported respiratory disease incidence in operations with PCR-detectable *Mycoplasma ovipneumoniae*, relative to uninfected operations. The x-axis shows the multiplicative change in each rate in the presence of *M. ovipneumoniae*, relative to the rate in the absence of *M. ovipneumoniae*. For instance, an interval extending from 1.2 to 2.6 would indicate that the corresponding rate is expected to be between 1.2 and 2.6 times higher in *M. ovipneumoniae*-positive flocks than in *M. ovipneumoniae*-negative flocks.

antibiotic use score (AIC = 28.4), operation size (AIC = 28.6), and disease burden score (AIC = 28.9) in addition to management type were competitive with management type alone, but these multivariate models were not selected because the individual effects were not significant ($p > 0.05$ delta-AIC > 2, Table S3).

3.5 Associations between animal health measures and *M. ovipneumoniae* presence

M. ovipneumoniae-infected operations often exhibited significantly less-productive vital rates than comparable operations without *M. ovipneumoniae*, after accounting for differences in management type and operation size (Figure 4). Birth rates among pregnant ewes were lower in *M. ovipneumoniae*-positive operations than in *M. ovipneumoniae*-negative operations (posterior mode for $e^\beta = 0.744$, 95% credible interval = [0.646, 0.870]; pseudo R-squared = 0.142, Table S4). Number of lambs born per pregnant ewe was higher in *M. ovipneumoniae*-positive operations than in *M. ovipneumoniae*-negative operations (posterior mode for $e^\beta = 1.055$, 95% credible interval = [1.024, 1.088]; pseudo R-squared = 0.082, Table S5), but *M. ovipneumoniae*-positive operations also had a higher proportion of lambs born dead than *M. ovipneumoniae*-negative operations (median proportion of full-term lambs born dead = 0.052 in *M. ovipneumoniae*-positive operations vs. 0.037 in *M. ovipneumoniae*-negative operations). Lamb survival to weaning was also lower, though not significantly so, in the presence of *M. ovipneumoniae* (posterior mode for $e^\beta = 0.922$, 95% credible interval = [0.833, 1.020]; pseudo R-squared = 0.171, Table S6). Overall ewe culling rates were higher in the presence of *M. ovipneumoniae* than in *M. ovipneumoniae*'s absence (posterior mode for $e^\beta = 1.178$, 95% credible interval = [1.036, 1.339]; pseudo R-squared = 0.147, Table S7). *M. ovipneumoniae* was associated with marginally higher lamb weaning weights (posterior mode for $\beta = 4.890$, 95% credible interval = [-0.953, 10.733], $p = 0.102$, Table S8, though this effect was not statistically significant at a 0.05 significance level, and may be due to unmodeled differences in twinning rates between *M. ovipneumoniae*-positive and -negative operations.

The data analyzed here did not provide conclusive evidence that culling rates increased for ewes, pre-weaning lamb, or weaned lambs at *M. ovipneumoniae*-positive operations over rates at *M. ovipneumoniae*-negative operations, after accounting for management type and operation size (posterior mode for $e^\beta = 4.903$, 95% credible interval = [0.917, 30.204], Table S9; pre-weaning lambs' posterior mode for $e^\beta = 4.333$, 95% credible interval = [0.720, 23.313], Table S10; weaned lambs' posterior mode for $e^\beta = 1.907$, 95% credible interval = [0.474, 6.615], Table S11).

3.6 Associations between vital rates and *M. ovipneumoniae* prevalence

WADDL and TEB's laboratory conducted additional testing on a subsample of 62 *M. ovipneumoniae*-positive operations to determine individual ewe prevalence. Higher prevalence (number of individuals testing PCR-positive for *M. ovipneumoniae* out of ten) was associated with lower full-term birth rates among pregnant ewes (posterior mode for $e^\beta = 0.709$, 95% credible interval = [0.572, 0.878], $p = 0.002$, pseudo R-squared = 0.877, Table S12) in models that also accounted for operation size and management type. Increased prevalence was associated with a decreased number of lambs born per ewe (posterior mode for $e^\beta = 0.758$, 95% credible interval = [0.726, 0.792], $p < 0.0001$, Table S13). Increased *M. ovipneumoniae* prevalence was associated with lower lamb survival to weaning among lambs born alive (posterior mode for $e^\beta = 0.455$, 95% credible interval = [0.391, 0.530], $p < 0.0001$, pseudo R-squared = 0.159, Table S14). Ewe culling rates increased significantly with increasing *M. ovipneumoniae* prevalence (posterior mode for $e^\beta = 1.722$, 95% credible interval = [1.484, 1.998], $p < 0.0001$, pseudo R-squared = 0.092, Table S15). There was no relationship between lamb weight at weaning and *M. ovipneumoniae* prevalence (posterior mode for $\beta = 0.784$; 95% credible interval = [-0.532, 2.101], $p = 0.908$, Table S16). Predicted changes in vital rates in response to the changes in *M. ovipneumoniae* prevalence reported in Table 2 are based on these models.

3.7 Consequences of *M. ovipneumoniae* infection on operation productivity

To evaluate the impact of infection on production, we compared two median-sized operations of 155 ewes each, one infected with *M. ovipneumoniae* and one uninfected. Of those ewes, we would expect 10.2% (15.8) to be culled in the *M. ovipneumoniae*-positive operation, and 9.0% (14.0) to be culled in the *M. ovipneumoniae*-negative operation, leaving 139.2 and 141.0 ewes, respectively. If all surviving ewes are bred at both operations, then the NAHMS data suggests that 141.0 (100%) of the ewes from *M. ovipneumoniae*-negative operation would deliver full-term lambs, but only 133.0 (95.4%) of the ewes from the *M. ovipneumoniae*-positive operation would do so. In the *M. ovipneumoniae*-positive operation, we would expect each bred ewe to produce 1.53 live lambs, for a total of 203.5 lambs born alive. In the *M. ovipneumoniae*-negative operation, that rate drops to 1.47 live lambs per ewe, for an expected total of 207.3 lambs born alive. Of those lambs, we would then expect 198.4 (95.7%) to survive to weaning at the *M. ovipneumoniae*-negative operation, but only 189.9 (93.3%) to survive to weaning at the *M. ovipneumoniae*-positive operation. Therefore, in aggregate, we would anticipate a reduction of roughly 8.5 lambs (4.3%) for an *M. ovipneumoniae*-positive operation, in comparison with an *M. ovipneumoniae*-negative but otherwise identical operation. If we finally assume that lambs can be sold for \$75-\$150 per head, this translates to an annual *M. ovipneumoniae*-imposed cost of \$637-\$1,275 for a median-sized flock.

4 Discussion

4.1 Conclusions

Evidence of *M. ovipneumoniae* infection was detected over 85% of the herded, pastured, and fenced domestic sheep operations that contributed biological samples to the first comprehensive survey of *Mycoplasma ovipneumoniae* in U.S. domestic sheep operations. Prevalences varied widely among the operations that received individual-level follow-up testing, from a single infected animal out of 10 sampled to universal infection in 10 sampled individuals. *M. ovipneumoniae*'s near ubiquity across domestic sheep operations means that even if infection has very small economic consequences at the individual level, those consequences likely accumulate over large numbers of animals and operations. This potentially imposes a heavy but unrecognized disease cost on domestic sheep production in the United States that contrasts with rare-but-devastating diseases like Scrapie which have the potential to accumulate high and visible economic burdens over a relatively small number of animals and operations (e.g., [33]). While there are relatively few survey studies of *M. ovipneumoniae* presence and prevalence in domestic sheep, our findings are on the same order as those of [2]. Those authors reported *M. ovipneumoniae* presence (as determined by PCR) in 8 of 11 tested flocks in the United Kingdom. In their case, however, seven of those flocks had a reported history of respiratory disease, and it is not entirely clear if flocks were chosen for testing on the basis of that history. Akwuobu et al. [34] reported 25 *M. ovipneumoniae* detections among 172 sample sheep from small ruminant operations in Nigeria, and Amin et al. [35] found a prevalence of 16.5% across 1047 samples from 139 flocks in Baluchistan, Pakistan. These individual-level prevalences are lower than those from our stratified sample, but production conditions are quite different.

Our estimate of *M. ovipneumoniae*'s burden on domestic sheep operations is subject to a number of sources of variation. In particular, we used vital rate estimates at the population level to estimate disease effects that actually operate at the individual level. Therefore, our individual-level burden estimates may be biased low, and are subject to additional variation associated with flock-level prevalences. *M. ovipneumoniae*'s burden was distributed across a variety of vital rates, as is true for other chronic ovine diseases like Johne's disease [36]. While the social costs of *M. ovipneumoniae*

are likely much lower than those of diseases with zoonotic potential, *M. ovipneumoniae* is nevertheless associated with substantial costs for production. Our estimate of *M. ovipneumoniae* burden may also be somewhat confounded by the overall higher disease burden exhibited by *M. ovipneumoniae*-positive operations. Further work is needed to separate *M. ovipneumoniae* effects from those of other pathogens. Nevertheless, losses of this magnitude may merit additional inquiry into management practices that could mitigate *M. ovipneumoniae* presence and prevalence per se. The ubiquity of *M. ovipneumoniae* infection in domestic sheep currently is a major challenge for control. Exploration into novel approaches that might be more widely applicable and cost-effective is a worth avenue for future work.

We were surprised by the positive effect of *M. ovipneumoniae* presence on the number of lambs born per ewe. Our analysis showed apparently divergent effects: while *M. ovipneumoniae* presence (binary) was associated with more lambs born per bred ewe, increased *M. ovipneumoniae* prevalence (continuous, and only among *M. ovipneumoniae*-positive operations) was associated with fewer lambs born per bred ewe. It is possible that some unmeasured factor associated with more lambs born per ewe was more common among *M. ovipneumoniae*-positive operations (potentially due to divergent management practices in positive and negative operations). When prevalence (only measured among infected operations) was considered, however, the coarse factor separating positive and negative operations may have been removed, allowing the weaker, yet still present *M. ovipneumoniae*-related vital rate shift to emerge. One plausible explanatory factor is mode of breeding. Smaller operations, which were also more likely to be *M. ovipneumoniae*-negative, may have used less reliable methods to determine how many of their ewes were bred than their larger counterparts. If small operators tended to over-estimate their numbers of “bred” ewes relative to large operators, that would inflate their denominator values in estimated number of lambs born per bred ewes, producing the apparently lower multiple birth rate reported here. Regardless of whether this is in fact the case, all vital rates presented here, and especially the multiple birth rate estimate, should be the topic of future investigation.

We saw significantly higher weaning weights in flocks that had any *M. ovipneumoniae* presence than in flocks where no *M. ovipneumoniae* was detected (i.e., a categorical variable where 1 = *M. ovipneumoniae*-positive and 0 = *M. ovipneumoniae*-negative), though this effect might be partially explained by differences in twinning rates between *M. ovipneumoniae*-positive and *M. ovipneumoniae*-negative operations. When we limited our inquiry to just *M. ovipneumoniae*-positive flocks, we saw no effect of prevalence on weaning weights. The direction of the effect was still positive (which is to say, higher prevalence was associated with higher weaning weight), but the standard error on that estimate was high enough that the estimated effect could have easily been due to sampling variation.

Operation size and management type were strongly related in the NAHMS dataset. This relationship did not have a strong deleterious effect on our model results; however, standard error estimates associated with size and operation effects did not change dramatically when models included only one or both variables as predictors. We present results from models containing both risk factors here, with the understanding that in practice, some operation sizes are only feasible under certain management types.

4.2 Project strengths and limitations

4.2.1 Scope of Inference

The NAHMS data presented here represent health patterns in operations with 20 or more ewes as of 2011, included in one of the 22 sampled states ([10] pg. 145). These are the strongest data available for studying prevalence of under-reported pathogens in domestic sheep throughout the United States, since invitation to participate in the NAHMS survey is determined through a rigorously designed, state-of-the-art sampling protocol. However, any sampling protocol that incorporates some element of participant choice (in this case, the invitees’ ability to decline the invitation to participate) can potentially depart from its intended stratification. As a consequence, and to avoid compounding even small biases due to participant choice, the sample data were not weighted back to the population totals in the present analysis.

Although our results are correlative in nature, our findings nonetheless suggest that *M. ovipneumoniae* may pose substantial yet under-recognized costs for domestic sheep production in the United States. Additional efforts to limit *M. ovipneumoniae* infection in domestic sheep operations could be of financial benefit to operators, and cost-effective methods to reduce infection burden should be explored.

4.2.2 Confounding with other respiratory diseases, and with region

The relationship between *M. ovipneumoniae* presence and vital rates and disease burden may be somewhat confounded by the generally higher overall disease burden exhibited by *M. ovipneumoniae*-positive operations. Further work is needed to separate *M. ovipneumoniae* effects from those of other pathogens, in particular ovine progressive pneumonia. Nevertheless, losses of this magnitude merit some additional inquiry about management practices that could mitigate *M. ovipneumoniae* presence and prevalence, along with those of other respiratory pathogens.

M. ovipneumoniae diagnostic testing continues to be an area of active refinement. The method initially used to test these samples is now known to cross-react on occasion with another Mycoplasma-like organism, but the re-testing we conducted using a modified test that eliminates that cross-reaction, together with the sequence confirmation of 180 test-positive samples from 30 randomly selected operations, confirms the lack of significant cross-reaction in the original testing for this study. Nevertheless, there is some possibility that additional refinement or recategorization of Mycoplasma spp. might produce different testing results in the future.

We omitted region information from our analysis, and this may have some consequences on our inferences. Region was confounded with management type and operation size, which were important predictors of *M. ovipneumoniae* prevalence and presence. We could not effectively separate region effects from management type effects within this dataset, so here we attribute the *M. ovipneumoniae* risk to management type. However, geographic effects should be addressed explicitly in follow-up work, if possible.

4.2.3 Within-operation sampling

We had limited information on how individual animals were selected for sampling within a flock. If *M. ovipneumoniae* transmission occurs predominantly within groups, and all sampled individuals were from the same group, then there might be a lack of independence among animals sampled within a particular operation. This correlation is unlikely to have introduced substantial bias into inferences based on *M. ovipneumoniae* presence, but could have produced biased results related to prevalence. In an effort to limit the role of that potential bias here, we chose not to emphasize overall prevalence rates, and focused instead on relationships between prevalence and various vital rates. Nevertheless, clustering should be explored in future investigations.

An additional issue associated with sampling is sample size within operations. These small sample sizes limited the precision of estimated relationships between *M. ovipneumoniae* prevalence and vital rates. For this reason, we primarily focused on the direction of estimated effects, and base our cost projections on an aggregate across all estimated vital rate relationships. Larger sample sizes from a carefully chosen set of focal operations would add valuable clarity to both the prevalence-vital rate relationships, and the cost projections.

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CONTAGIOUS ECTHYMA IN BIGHORN SHEEP AND MOUNTAIN GOAT IN WESTERN CANADA 1

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CONTAGIOUS ECTHYMA IN BIGHORN SHEEP AND MOUNTAIN GOAT IN WESTERN CANADA^[1]

W. M. SAMUEL,^[2] G. A. CHALMERS,^[3] J. G. STELFOX,^[4] A. LOEWEN,^[5] and J. J. THOMSEN^[6]

Abstract: Contagious ecthyma (CE) is reported in bighorn sheep (*Ovis c. canadensis*) from several national parks in western Canada and in mountain goat (*Oreamnos americanus*) from Kootenay National Park, British Columbia. (This is the first report of CE in mountain goat.) Diagnosis was based on clinical signs, histopathology, transmission experiments and the demonstration of a poxvirus with the electron microscope. The infection was transmitted from wild to domestic goat, but not to domestic sheep.

Most infections, some of them severe, were found in lambs and kids. Clinical signs of disease were similar to those seen in domestic sheep and goats. General body condition was poor and animals had difficulty feeding normally.

All infected herds had prolonged contact with areas where salt was provided artificially (i.e., salt blocks, highways and campgrounds). Fewer infected sheep were observed annually when salt blocks were removed from Jasper National Park.

INTRODUCTION

Contagious ecthyma (CE) is a viral disease of domestic sheep and goats from many countries and is known by a variety of descriptive terms including sore mouth, contagious pustular dermatitis, scabby mouth, infectious labial dermatitis, and orf.⁴ The clinical disease is characterized by proliferative, crusted and sometimes pustular lesions of the lips, muzzle and occasionally the udder, feet and vulva.⁶ Little is known about CE in wild animals; it has been reported only from bighorn sheep, chamois, thar and steinbok.⁶ Connell² and Blood¹ reviewed the history of CE to 1965 in bighorn sheep from four national parks in western Canada; no mortality was attributed directly to CE, but several cases of the disease were observed in lambs. This report summarizes recent developments concerning CE in bighorn sheep (*Ovis c.*

canadensis) from western Canada and documents the disease in mountain goats (*Oreamnos americanus*).

HISTORY

CE has been observed in bighorn sheep from western Canada since 1953.² Sixteen infected sheep comprising 11 lambs and five ewes, were collected from Jasper, Banff, Kootenay and Waterton Lakes National Parks since 1953¹ (Stelfox, unpub.). Most animals were in debilitated condition, with severe labial lesions. Many other sheep, particularly lambs, were observed with lesions suggestive of CE.

Since 1959, 58 suspected cases which includes 49 lambs have been observed in Jasper National Park. The number of suspected cases remained low (≤ 3) each year until 1966, when 15 of 76 sheep in

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the Mile 3 band (see Fig. 1) and two other sheep from other bands were infected. In the Disaster band which has been observed closely since 1968, 51 of approximately 80 sheep were examined in February, 1968; one of 14 lambs was infected. Two of four lambs in a group of 12 were infected in September, 1970. One of eight lambs in a band of 42 sheep was infected in December, 1970. The number of presumptive cases observed annually from 1967 to June 7, 1973, in Jasper was 6, 4, 8, 4, 4, 2, and 0 respectively.

Of the 58 cases observed in Jasper National Park, 52 were seen from September to December, five from January to March and one infected lamb was observed in mid-July. None were observed in August and 10 in September.

In all cases observed in the national parks,¹ sheep have had prolonged contact with areas where salt was provided artificially (i.e., salt blocks, highways and campgrounds).

Lesions suggestive of CE have been observed in bighorn sheep east of Banff National Park,¹ in the Sheep River area in 1972 and 1973 (Horejsi, pers. comm.) (Fig. 1), and in Montana.¹ Six of 19 lambs were infected in a band of 43 sheep trapped from January to March, 1972, at Sheep River. In June-July 1972, three infected lambs were observed; during the winter, 1973, eight of 19 lambs were infected. None of the infections was considered severe (Horejsi, pers. comm.). Sheep visit artificial sources of salt between September and May of each year in the Sheep River area.

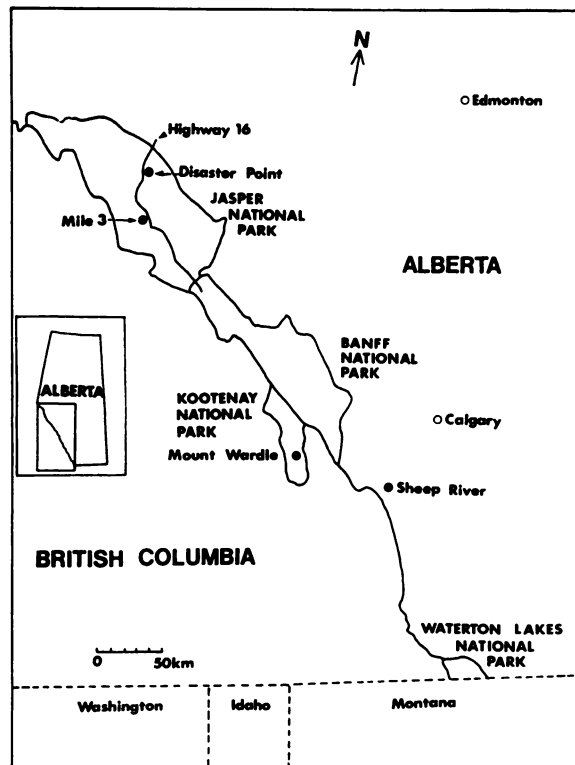


FIGURE 1. Location of western National Parks and several infected bands of bighorn sheep and mountain goat.

CE has been observed in goats from Mount Wardle since 1974 (Sime, pers. comm.). Four adults, two males and two females, were found moribund in January 1947 with severe lesions on the lips and muzzle. All animals died within 1 month of capture. Approximately 90 adults produced 18 kids on Mount Wardle in 1967, and at least six kids were observed with clinical signs of the disease (DeBock and Winkler, pers. comm.). Three of the kids with severe lesions of the lips and muzzle were found moribund in July. A 9-month old male with severe labial lesions was collected on Mount Wardle in February 1972; it weighed 12 kg. There were no reports of CE infection in 1973. Four of five mineral licks used by goats on their winter range are semi-natural.³ No infected sheep have been observed on Mount Wardle (Stelfox, unpub.), but CE-infected sheep have been collected from other parts of Kootenay National Park.¹

CASE REPORT — SHEEP

Two emaciated animals, a ewe and lamb, with severe lesions of the muzzle and lips, were observed and subsequently killed in Waterton Lakes National Park (Fig. 1) in April, 1971. The ewe was emaciated and weighed only 26 kg.

Gross examination of the lamb revealed numerous irregular, confluent, raised, crusted, dark brown to black lesions located along the margins of the lips including the commissures, and on the muzzle and nose. Most lesions were dry, but pustules were also present. Lesions were particularly severe in the ewe (Figs. 2-3) and were characterized by markedly thickened, edematous lips covered by reddish-brown, fissured, crusted proliferative epithelium (Fig. 2). Several pale, circumscribed, raised lesions of various diameters were observed on the dorsum of the tongue; smaller red papilloma-like proliferations, some of them perdunculated, were evident along the anterior leading edge. Numerous small pink proliferative lesions were observed clustered on the gingival mucosa at the base of the incisiform teeth

(Fig. 3); blackened proliferations could also be found on the dental pad, hard palate and at the base of the molars.

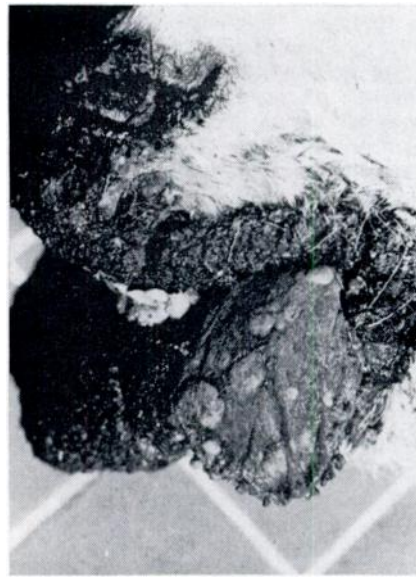


FIGURE 2. Contagious ecthyma in a bighorn sheep. Lesions on the nose, lips and tongue.



FIGURE 3. Contagious ecthyma in a bighorn sheep. Lesions on the gingival mucosa.

CASE REPORT — GOAT

A severely infected moribund mountain goat kid (Fig. 4) was found by park personnel at the base of Mount Wardle in Kootenay National Park (Fig. 1) on March 1, 1972, and was transported to the University of Alberta on March 9, 1972. The kid weighed 13.4 kg on arrival and improved during its second week, following treatment for suspected nutritional myopathy. Although appetite was good, prehension and mastication were difficult and the animal's condition continued to deteriorate. At the time of death on April 3, 1972, the kid weighed 11.5 kg.

Dry, brown, proliferative lesions were prominent on the markedly edematous hyperemic lips, but diminished in severity toward the commissures (Fig. 4). Lesions of the oral cavity were moist, reddish-brown and in certain sites, intensely hyperemic. A large proliferative mass on the gingiva immediately posterior to the incisiform teeth apparently interfered with the normal placement of the tongue.

Examination of the remainder of the carcass revealed numerous pulmonary

abscesses along the ventral borders of the diaphragmatic lobes, with extensive fibrous adhesions to the ribs and diaphragm. As well, abscesses were observed on the pericardial sac and in the abdominal cavity, with many fibrous adhesions between the diaphragm and the rumen. Bacteriologic examinations of pulmonary abscesses yielded *Corynebacterium pyogenes* and *Pasteurella multocida*. The following helminths were found: 790 *Ostertagia occidentalis* and *Marshallagia marshalli* (abomasum); 249 *Nematodirus maculosus*, several *Thysanosoma actinioides*, many unidentified Anoplocephalidae (small intestine); 123 *Trichuris oreamnos* (caecum); and 3 *Protostrongylus rushi* (lungs).

HISTOPATHOLOGY

Selected oral and labial lesions were trimmed to 2-3 mm thickness, fixed in 10% neutral buffered formalin, processed in several changes of ethyl alcohol, xylene and paraffin, cut at 6 μ m and stained with haematoxylin and eosin (H & E).

Histopathologic lesions were similar in both the sheep and goat. Hyperplastic non-pigmented squamous epithelium, often thrown upward into folds and long spike-like projections with marked hyperkeratosis, some parakeratosis and microabscesses in the keratinized layer, characterized the lesions. Edema was prominent at the tips of epithelial folds, and in the keratinized layer, pools of serous fluid separated by thin keratin strands had a characteristic multilocular appearance. Long interconnecting rete pegs extended into the dermis, in which the proliferating connective tissue was canalized by dilated erythrocyte-filled capillaries. Inflammatory cells in the dermis consisted mainly of dense accumulations of lymphocytes, with lesser numbers of plasmacytes, neutrophils and some eosinophils.

Numerous swollen, vacuolated epithelial cells, best appreciated in the immediate vicinity of hair follicles, each contained one or more oval or round eosinophilic cytoplasmic inclusions.



FIGURE 4. Lesions of contagious ecthyma on the lips of a mountain goat.

VIRAL ISOLATION AND TRANSMISSION ATTEMPTS

Lesions were removed from the lip of the mountain goat and stored in refrigerated sterile buffered glycerine. Tissue was washed 3 times in saline and ground in a mortar to prepare a 10% suspension based on wet tissue weight. Transmission was attempted by spreading the suspension on a shaved, lightly scarified area of the flanks of two domestic sheep and one domestic goat. Typical vesicles and pustules characteristic of CE began to appear at the site of inoculation on the goat at 21 days. Lesions did not appear on the sheep. Typical CE viral particles⁷ were demonstrated by electron microscopy, using negative staining with 2% pH 7.0 phosphotungstate, from extracts of swabs from the wild and domestic goats. The lesions on the domestic goat began to heal 30-35 days after inoculation; the inoculation site was clear by 40 days. CE has been transmitted successfully from bighorn to domestic sheep previously.^{1,2}

DISCUSSION

CE has been reported rarely from animals other than domestic sheep and goat. The present data and those of Blood¹ indicate an important large focus of CE in bighorn sheep in British Columbia and Alberta. The disease is apparently restricted to certain mountain goat populations, having been reported only from Mount Wardle in Kootenay National Park, British Columbia. Many bands of uninfected goats have been observed in and near western Canadian national parks (Stelfox, Loewen, unpub.,

and Cowan, Hebert and others, pers. comm.).

Several sheep and goats severely infected with CE have been found dead or moribund. It is evident that prehension and mastication are altered if the lips, muzzle and buccal membranes are severely affected. These observations suggest strongly that CE, possibly in combination with high numbers of helminths (Samuel, unpub.), bacterial infections and a low plane of nutrition, is an important mortality factor in certain bands of wild sheep and goats. In animals with mild infections, mortality due to CE *per se* is probably negligible. Horejsi (pers. comm.) noted no difference between the feeding behavior of infected and uninfected lambs at Sheep River.

The role of artificial sources of salt in the epidemiology of CE is unclear. For many years, salt blocks were placed strategically in the parks to concentrate sheep and other ungulates for observation by tourists. A dangerous zoonotic component of this policy was the observation of children breaking off and licking pieces from salt blocks used by infected bands of sheep (Stelfox, unpub.). This distribution of salt, predominately along the major road in Jasper Park, was discontinued between 1966 and 1970. However, sheep continued to return to the lick areas and eat large quantities of soil or to receive feed from tourists. Bands at Mile 3 and Disaster obtain limited quantities of salt from tourists and highway sources. At least one uninfected band of 60 sheep near the Jasper-Banff boundary has had access to block salt in the past and to heavy highway salting at the present time.

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RECOMMENDATIONS FOR Domestic Sheep and Goat Management in Wild Sheep Habitat



Prepared by the
Wild Sheep Working Group

Western Association of
Fish and Wildlife Agencies

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Executive Summary

Executive Summary

Although the risk of disease transmission from domestic sheep or goats to wild sheep is widely recognized, a unified set of management recommendations for minimizing this risk has not been adopted by responsible agencies. These Western Association of Fish and Wildlife Agencies (WAFWA) recommendations were produced to help state, provincial, and territorial wild sheep managers, federal/crown land management agencies, private landowners and others take appropriate steps to eliminate range overlap, and thereby, reduce opportunities for transmission of pathogens to wild sheep.

Transmission of *Mannheimia haemolytica* from domestic sheep to bighorn sheep was irrefutably demonstrated by Lawrence et al. (2010) and provides justification sufficient for preventing range overlap and potential association of domestic sheep and goats with wild sheep. The higher the

conservation value of a wild sheep population (e.g., federally or state listed, “sensitive species” status, native herds, transplant source stock, herds in areas with no history of domestic livestock presence), the more aggressive and comprehensive wild sheep and domestic sheep or goat separation management strategies should be.

Practical solutions will be difficult, if not impossible to achieve until the risk of disease transmission from domestic sheep or goats to wild sheep is acknowledged by those responsible for wildlife and agricultural management. All parties benefit when risk is assessed and actively managed to minimize the potential for transmission of pathogens. The recommendations contained within this report are intended to help achieve that objective to benefit all sectors and are summarized as follows:

WAFWA agencies should:

- (1) assess wild sheep conservation value/status and complete risk assessments of interspecies contact in a meta-population context;
- (2) remove wild sheep that have likely associated with domestic sheep or goats and develop a policy to promptly respond to wild sheep wandering from occupied wild sheep ranges;
- (3) thoroughly explore demographic consequences of translocations and conduct appropriate analyses of habitat suitability and risk of disease transfer prior to implementing any translocations;
- (4) coordinate with other agencies, land owners and stakeholders regarding management of domestic sheep or goats on or near ranges occupied by wild sheep;
- (5) fully consider the risk of disease transmission when issuing or commenting on permits/regulations associated with private lands used for domestic production; and
- (6) develop educational materials and outreach programs to interpret the risk of association between wild sheep and domestic sheep or goats.

Land management agencies should:

- (1) reduce risk of association by eliminating overlap of domestic sheep or goat allotments or grazing permits/tenures within wild sheep habitat;
- (2) ensure that annual operating instructions or their equivalent include measures to minimize domestic association with wild sheep and confirm appropriate methods to remove stray domestic sheep or goats; and
- (3) manage wild sheep habitat to promote healthy populations in areas without domestic sheep or goats.

Wild sheep conservation organizations should:

- (1) assist with educational/extension efforts to all parties;
- (2) negotiate alternatives and incentives for domestic sheep or goat grazers on public land to find alternatives to wild sheep habitat; and
- (3) advocate for and support research concerning disease and risk associated with domestic sheep and goats in proximity to wild sheep.



Photo by: David Wetzel (Texas Bighorn Society)



Photo by: Tom Carlsen (MDFWP)



Introduction

Domestic sheep and goat permittees/owners should:

(1) implement best management practices (BMPs) to prevent straying by domestic sheep or goats; and (2) establish protocols to respond to straying.

Private landowners should:

(1) educate themselves and work with wild sheep managers and advocates to support effective separation through a variety of site-specific mitigation measures; and (2) promptly report the potential or actual association between domestic sheep or goats and wild sheep.

Introduction

In January 2007, the Western Association of Fish and Wildlife Agencies (WAFWA), comprised of 23 state and provincial wildlife agencies from the western United States (U.S.) and western Canada, established a Wild Sheep Working Group (WSWG) to develop a report titled, “Recommendations for Domestic Sheep and Goat Management in Wild Sheep Habitat” (WAFWA 2007). Unanimously endorsed by WAFWA Directors in July 2007, that report provided recommendations to which state, provincial and federal agencies could tier their management actions. In August 2007, the report was forwarded to the heads of the U.S. Forest Service (USFS), Bureau of Land Management (BLM), National Park Service, U.S. Fish and Wildlife Service, Bureau of Reclamation, and Department of Defense. In July 2010, the report was revised (WAFWA 2010c) and has represented the official position of WAFWA on the management of domestic sheep and goats and wild sheep.

Scientific literature that has become available since July 2010 has been incorporated into this document to ensure that the recommendations contained herein remain current and robust, but the basic purpose, scope, and principles of the document remain unchanged. Additional editorial modifications are intended to improve the readability of the document. Information contained in this report is provided to assist BLM and USFS leadership with development of a unified policy addressing the grazing of domestic sheep or goats in wild sheep habitat on lands under the administration of those agencies. In addition, this document is intended to assist state, provincial, and territorial wild sheep managers, federal/crown land management agencies, private landowners and others take appropriate steps to eliminate range overlap, and thereby, reduce opportunities for transmission of pathogens to wild sheep. This revision was approved by the WAFWA Directors March 29, 2012, and supersedes all previous versions.

In this paper we do not review and synthesize all available literature or evidence pertaining to the issue of disease transmission among bighorn sheep and domestic sheep and goats. We do, however, include relevant citations, results,



Photo by: Earl Nottingham (TPWD)



Photo by: Dr. Peri Wolff (NDOW)

literature, or analyses published since completion of our previous reports (WAFWA 2007, 2010c). We provide reasonable and logical recommendations based on the best available information to help achieve effective separation between wild sheep and domestic sheep or goats. We recognize it is impossible to achieve zero risk of contact or disease transmission; however, we also recognize there are many ways to reduce the probability of association between these species and, thereby, lower the overall risk of epizootics occurring in populations of wild sheep.

Background



Photo by: David Wetzel (Texas Bighorn Society)



Photo by: Mike Pittman (TPWD)



Photo by: Brett Wiedmann (NDGFD)

Background

Throughout substantial portions of their range, bighorn sheep (*Ovis canadensis*) experience periods when populations are depressed; those episodes generally are associated with epizootics of respiratory disease (Ryder et al.1994). Diseases have contributed to the decline of bighorn sheep populations in much of western North America (Beecham et al. 2007, CAST 2008) and many native herds declined to less than 10% of historical size. According to historical accounts, such declines coincided with the advent of domestic livestock grazing on ranges occupied by bighorn sheep (Warren 1910, Grinnell 1928, Schillinger 1937, Honess and Frost 1942, CAST 2008). Epizootics among native bighorn herds were reported in various locations following European settlement and establishment of domestic livestock grazing throughout the central and southern Rocky Mountains. These observations may reflect the introduction of novel bacterial pathogens (including some strains of *Pasteurella [Mannheimia]* spp.) to naïve bighorn populations beginning in the late 1800s (Grinnell 1928, Skinner 1928, Marsh 1938, Honess and Frost 1942, Miller 2001).

Over the past 30 years, increasing evidence has underscored the potential risk of disease transmission from domestic sheep or goats to wild sheep (McQuivey 1978, Hunt 1980, Jessup 1982, Foreyt and Jessup 1982, Goodson 1982, Onderka and Wishart 1984, Jessup 1985, Black et al.1988, Coggins 1988, Festa-Bianchet 1988, Onderka and Wishart 1988, Onderka et al.1988, Schwantje 1988, Callan et al.1991, Coggins and Matthews 1992, Foreyt 1994, Foreyt et al. 1994, Cassirer et al.1996, Foreyt and Lagerquist 1996, Martin et al. 1996, Coggins 2002, Rudolph et al. 2003, Jenkins et al. 2007, Rudolph et al. 2007, George et al. 2008, Jeffress 2008, Lawrence et al. 2010).

Moreover, a number of recent risk assessments and reviews (Beecham et al. 2007, CAST 2008, Baumer et al. 2009, USAHA 2009, WAFWA 2009, Croft et al. 2010, USDA Forest Service 2010a, b; Wehausen et al. 2011), conservation management strategies or plans (Colorado Division of Wildlife 2009, Montana Department of Fish, Wildlife, and Parks 2009), modeling exercises (Clifford et al. 2009, Cahn et al. 2011), and many wildlife biologists and wildlife veterinarians (Gross et al. 2000, Singer et al. 2000, Dubay et al. 2002, Epps et al. 2004, Garde et al. 2005, Jansen et al. 2006, Foreyt et al. 2009) have focused on risks associated with contact between wild sheep and domestic sheep or goats. Many of the aforementioned investigators and participants in workshops conducted throughout the western US (California, Arizona, Utah, and Idaho),

have recommended temporal or spatial separation of domestic sheep or goats from wild sheep to reduce the potential for disease in the latter.

Disease Transmission

Although domestic animals have been selected for their ability to live at high densities and for their resilience to infectious diseases (Diamond 1997), two-way transmission of certain diseases (e.g., paratuberculosis, some enteric pathogens and parasites) between wild sheep and domestic sheep or goats in shared habitats can occur (Garde et al. 2005). However, the most important and ecologically significant transmission in this context is from domestic sheep or goats to wild sheep.

Winter 2009-2010 bighorn sheep pneumonia die-offs (totaling an estimated 880 bighorns) in Montana, Nevada, Washington, Utah, and Wyoming have reduced bighorn numbers in at least 9 herds, either through direct mortality or agency removal (i.e., “culling”) of bighorn sheep exhibiting symptoms of respiratory infections (Edwards et al. 2010, WAFWA 2010b). Domestic sheep and goats were known to occur within or near occupied bighorn sheep ranges and within normal bighorn movement zones, and association between wild sheep and domestic sheep or goats is known to have preceded at least one of these die-offs, was likely in 2 others, and was possible in 4 more (WAFWA 2010b).

Die-offs of wild sheep populations and individual animals have occurred in the absence of reported association with domestic sheep or goats (Aune et al. 1998, UC-Davis 2007). However, when contact between wild sheep and domestic sheep or goats has been documented, the pattern and severity of die-off is typically greater than when otherwise is the case (Onderka and Wishart 1984, Martin et al. 1996, Aune et al. 1998, George et al. 2008).

It is generally acknowledged (Garde et al. 2005, CAST 2008) that thimhorn sheep (*Ovis dalli* spp.) in Alaska and northwestern Canada are likely naïve to exposure to many organisms commonly carried by domestic species, compared to wild sheep occurring in southern Canada and the continental U.S. Until this is confirmed and the effects of exposure to infectious organisms are clearly understood, it is essential that no association occurs between thimhorn sheep and domestic sheep or goats.



Photo by: Donny Martorello (WDFW)



Photo by: Ernie Finch

Effective Separation



Photo by: John Kanta (SDGFP)



Photo by: Ted Borda (Borda Land & Sheep Company)



Photo by: Ted Borda (Borda Land & Sheep Company)

Effective Separation

WAFWA defines “Effective Separation” as spatial or temporal separation between wild sheep and domestic sheep or goats to minimize the potential for association and the probability of transmission of diseases between species. WAFWA advocates that effective separation should be a primary management goal of state, provincial, territorial and federal agencies responsible for the conservation of wild sheep, based on evidence that domestic sheep or goats can transfer pathogens to wild sheep. Literature (reviewed by Wehausen et al. 2011) and experimental evidence (Lawrence et al. 2010) support the goal that domestic sheep or goats should not concurrently occupy areas where conservation of wild sheep is a clearly stated management goal.

Effective separation does not necessarily require removal of domestic sheep or goats in all situations. However, the option of removing domestic sheep or goats should be included in an array of alternatives available to address this issue. In fact, some collaborative working groups (USAHA 2009) have recommended domestic goats not be allowed to graze in occupied bighorn sheep habitat because of their gregarious nature and tendency to wander. We are aware of the continuing debate and discussion (CAST 2008, USAHA 2009) between wildlife advocates and some domestic sheep or goat industry proponents and resource managers regarding the credibility or scientific merit of past findings; that debate is founded largely on criticisms of experimental design or rigor, and limitations of drawing inferences about natural disease events when compared to controlled experiments in confined settings. However, it is WAFWA's collective opinion that enough is known about potential pathogen transmission from domestic sheep or goats to wild sheep that efforts toward achieving effective separation are necessary and warranted.

Reducing risk of disease transmission on the landscape by minimizing or preventing association between wild and domestic sheep or goats is a key management strategy for WAFWA agencies (e.g., Colorado Division of Wildlife 2009, Montana Department of Fish, Wildlife and Parks 2009). Legislation in Utah (House Bill 240 Supplement, 2009), Wyoming (Senate Enrolled Act No. 30, 2009) and Idaho (Senate Bill 1232 amended, 2009) provides direction, authority and responsibilities for addressing feral or stray livestock that pose a disease transmission risk. Further, recent court rulings (e.g., U.S. District Court, Idaho Case 09-0507-BLW) have mandated separation between domestic sheep or goats and bighorn sheep, including mandatory non-use of grazing allotments where effective separation could not be assured.



Effective Separation

Principal federal land management agencies in the western U.S., BLM and USFS, continue to review, revise, and update policies on the management of domestic sheep or goats in wild sheep habitat (USDI BLM 1992, 1998, 2010; USDA Forest Service 2009). Additionally, several administrative units of the USFS (Northern Region, Rocky Mountain Region, Southwest Region, Intermountain Region, and the Pacific Southwest Region) have designated bighorn sheep as a “Sensitive Species,” thereby mandating special management emphasis. This includes: thorough reviews and analyses of management actions that could affect populations of bighorn sheep or their habitat to ensure their viability and to preclude demographic trends that would result in the need for Federal listing.

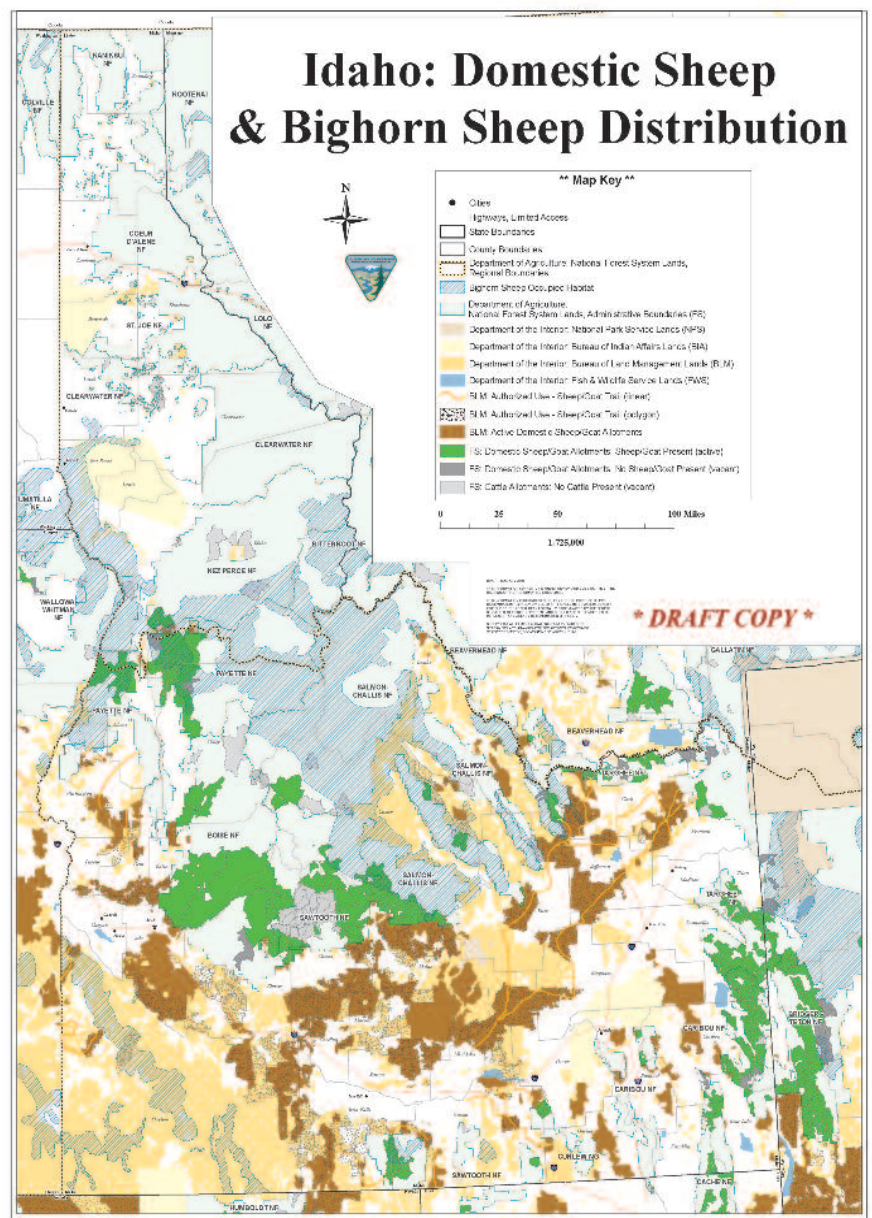
An interagency GIS-based decision-support tool and GIS coverage maps that overlay current bighorn sheep distribution with vacant and active domestic sheep or goat grazing allotments and trailing routes were finalized for 14 western states (WAFWA 2010a). These maps identify areas where association between domestic sheep or goats and bighorn sheep could occur on, or adjacent to, lands managed by BLM or USFS, and also identify areas that could provide spatial separation. The maps further provide a context for national policy development, and help identify situations where proactive management is necessary to minimize risk of association. Although risk of disease transmission from domestic sheep or goats to wild sheep is widely acknowledged by wildlife and land management agencies, a unified set of management guidelines for minimizing this risk has not yet been adopted.

In some cases, results of contact between domestic sheep or goats and wild sheep have been severe enough to endanger entire populations of the latter. In Idaho, legislation (Senate Bill 1232 amended, May 2009) mandated collaboration between the Idaho Department of Fish and Game and domestic sheep grazing permittees that identified BMPs to achieve effective separation between domestic sheep and wild sheep on both public and private lands. In specific situations, implementation of BMPs could lead to a reduced risk of association. In particular, BMPs implemented in open, gentle terrain where domestic sheep or goats can be easily controlled and monitored can reduce risk of association (Schommer 2009). Nevertheless, BMPs that work in one situation may not work in other situations (Schommer 2009).

Consequently, we recommend that managers take appropriate steps to minimize opportunities for association and, thereby, the potential for disease transmission in all situations.



Photo by: Bighorn Institute



Provided by: Chans O'Brien (USFS)

Management Recommendations

Management Recommendations

The recommendations that follow can be applied to state, provincial, and territorial wildlife agencies, federal/crown land management agencies, wild sheep conservation organizations, domestic sheep or goat producers or permittees, and private landowners, and have been strategically assigned to logical categories. It is imperative, however, that readers recognize these recommendations typically apply to multiple parties, and that they further recognize that a multi-disciplinary and collaborative approach will produce the best outcomes, both for wild sheep and for producers or permittees. Definitions of various terms used throughout this document are provided in Appendix A.

Although these recommendations have been developed by a working group largely comprised of wildlife agency personnel, cooperation between numerous concerned parties is critically important to deriving on-the-ground solutions (USAHA 2009, Wild Sheep Foundation 2011). Among these are state, provincial, and territorial wildlife agencies; federal/crown land management agencies; First Nation or tribal representatives; domestic sheep or goat producers or grazing permittees; agricultural industry representatives; wild sheep conservation organizations; environmental groups; academic institutions; and interested individuals. As a result of information contained herein, it is our hope that collaborative discussions will occur and that those discussions yield results in the form of innovative and collaborative site-specific delivery of programs such as the British Columbia Wild/Domestic Sheep Separation Program and the Wyoming Statewide Domestic Sheep/Bighorn Sheep Interaction Working Group.

Many anthropogenic and environmental factors (CAST 2008) influence the demographics and viability of wild sheep populations. Some factors affecting wild sheep population performance can be managed while others cannot. Nevertheless, the guiding principle of our effort has been “to seek effective separation” between wild sheep and domestic sheep or goats. There is no “one size fits all” risk assessment of respiratory disease transmission between wild sheep and domestic sheep or goats. However, a comprehensive risk assessment (qualitative and quantitative) is a critically important component for managing the potential for disease transmission.

We recommend that wild sheep managers design and implement management strategies by taking the first step of assessing and prioritizing conservation value and relative importance of wild sheep populations. The greater the conservation value and the greater the risk of association with domestic sheep or goats, the more aggressive and comprehensive a strategy to ensure effective separation should be. To ensure that is the case, we offer the following:



Photo by: Rebecca Barboza (CDFG)



Photo by: Larry Kruckenberg

Management Recommendations

RECOMMENDATIONS TO WAFWA AGENCIES

■ Historic and suitable but currently unoccupied wild sheep range should be identified, evaluated, and compared against currently-occupied wild sheep distribution and existing or potential areas where domestic sheep or goats may occur.

■ Risk assessments should be completed at least once per decade (more often if warranted) for existing and potential wild sheep habitat. These assessments should specifically identify where and to what extent wild sheep could interface with domestic sheep or goats, and the level of risk within those areas.

■ Following completion of site or herd-specific risk assessments, any translocations, population augmentations, or other restoration and management strategies for wild sheep should minimize the likelihood of association between wild sheep and domestic sheep or goats. Agencies should:

- Avoid translocations of wild sheep into areas with no reasonable likelihood of effective separation from domestic sheep or goats.
- Re-evaluate planned translocations of wild sheep to historical ranges as potential conflicts, landscape conditions, and habitat suitability change.
- Recognize that augmentation of a wild sheep herd from discrete source populations poses a risk of pathogen transfer (CAST 2008) and thus, only use source stock verified as healthy through a proper health assessment (WAFWA 2009) for translocations. Source herds should have extensive health histories and be regularly monitored to evaluate herd health. Wild sheep managers should evaluate tradeoffs between anticipated benefits such as demographic, behavioral and genetic interchange, and the potential consequences of mixing wild sheep from various source herds.
- Develop and employ mapping or modeling technology as well as ground based land use reviews prior to translocations to compare wild sheep distribution and movements with distribution of domestic sheep or goats. If a translocation is implemented and association with domestic sheep or goats occurs, or is likely to occur beyond an identified timeframe or pre-determined geographic area, domestic sheep or goat producers should be held harmless.

■ The higher the risk of association between wild sheep and domestic sheep or goats, the more intensively wild sheep herds should be monitored and managed. This is particularly important when considering “new” vs. “augmented” wild sheep populations.

- Site-specific protocols should be developed when association with domestic sheep or goats is probable. For example, decisions concerning percentage of translocated wild sheep that must be radio-collared



Photo by: Mike Cox (NDOW)



Photo by: Chase Fountain (TPWD)

Management Recommendations

for achieving desired monitoring intensities should in part, be based upon the subsequent level of risk of association with domestic sheep or goats.

- Intensive monitoring provides a mechanism for determining proximity of wild sheep to domestic sheep or goats and for evaluating post-release habitat use and movements.
- Budgets for wild sheep translocation projects should include adequate funding for long-term monitoring.

■ Wild sheep managers should identify, analyze, and evaluate the implications of connectivity and movement corridors between largely insular herds comprising a meta-population against opportunities for increased association with domestic sheep or goats. Analyses should include distribution and continuity (Mack 2008) among populations of wild sheep and the anticipated frequency of movement among or within wild sheep range. In doing so, the benefits

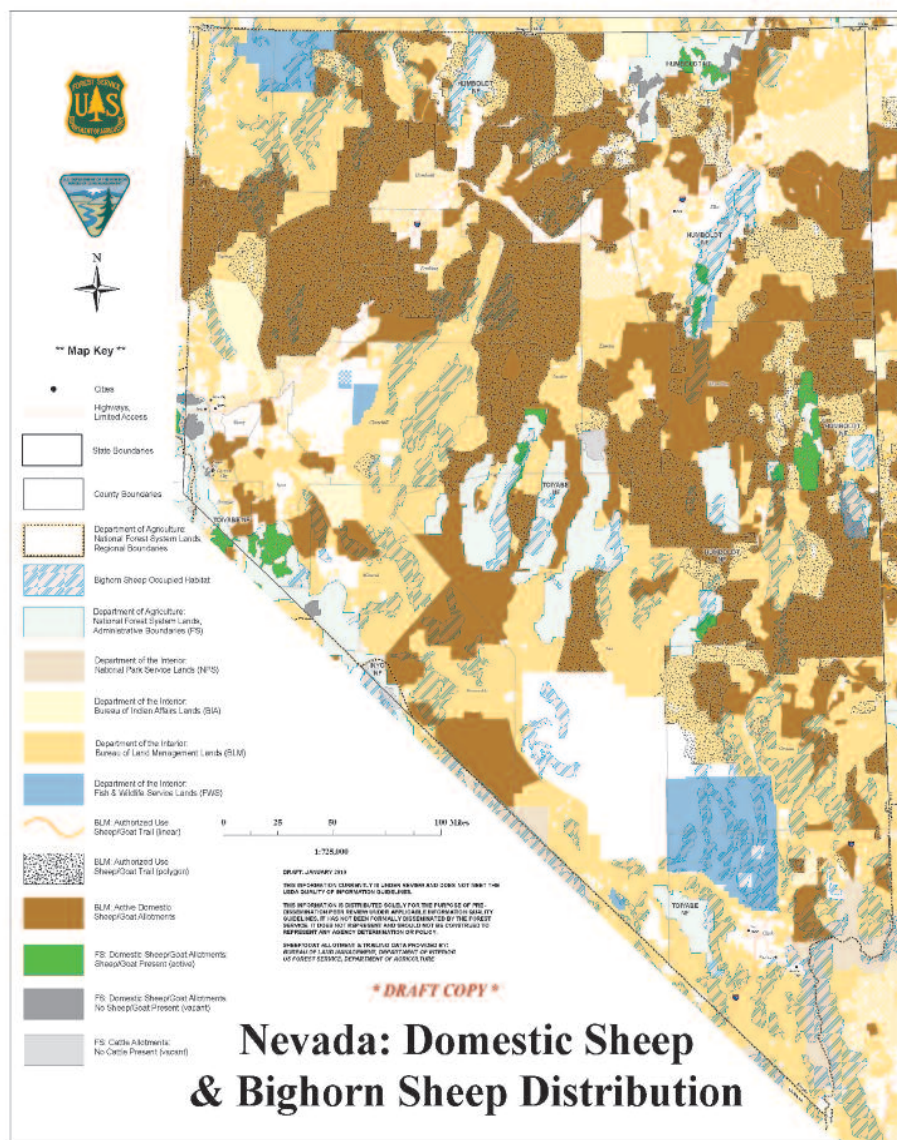
of genetic interchange and its resultant implications for population viability, must be weighed against the risks of disease transmission (Bleich et al. 1990), especially if dispersing or wandering wild sheep could travel across domestic sheep or goat grazing allotments or trailing routes, private land holdings or other areas where the potential transfer of endemic pathogens from an infected wild herd to a naïve herd could occur.

■ Removal of wild sheep known, or suspected to have closely associated with domestic sheep or goats is considered to be an effective management tool. Atypical movements by wild sheep can heighten risk of association with domestic sheep or goats. Additional measures to achieve effective separation should be implemented if such association occurs. However, removal of wild sheep from occupied, normally-anticipated wild sheep range is not always the best management option.

Continuous risk of association exists during active grazing seasons when domestic sheep or goats are grazed within normally-anticipated wild sheep range. Thus, removal of individual wild sheep is an ineffective method for maintaining separation, and has potentially negative consequences for population viability. Removal of wild sheep should occur only after critical evaluation and further implementation of measures designed to minimize association and enhance effective separation.

■ Wild sheep populations should have pre-determined population objectives, and should be managed at agreed-upon densities to minimize the potential for dispersal. Because some dispersal occurs regardless of population density, some risk of association is always present if domestic sheep or goats are within range of dispersing wild sheep.

■ Agencies should develop a written protocol to be implemented when association between wild sheep and domestic sheep or goats is confirmed. Notification requirements, appropriate response and post-contact monitoring options for both domestic sheep and goats and dispersing or wandering wild sheep should be included. Moreover, wildlife agencies should collaborate with agricultural agencies, land management agencies, producers and permittees, grazing industry representatives,



Provided by: Chans O'Brien (USFS)



Management Recommendations

and wild sheep advocates to develop an effective, efficient, and legal protocol to be implemented when feral or abandoned domestic sheep or goats threaten to associate with wild sheep but for which no owner can be identified. Written protocol examples are provided in Appendix B (British Columbia Fish, Wildlife and Habitat Management Branch) and Appendix C (Wyoming Game and Fish Department).

- Wildlife agencies should develop databases as a system to report, record, and summarize association between wild sheep and domestic sheep or goats and its outcome; the WAFWA WSWG website (<http://www.wafwa.org/html/wswg.shtml>) would be a logical host. Further, wildlife managers and federal/crown land managers should encourage prompt reporting by the public of observed proximity between wild sheep and domestic sheep or goats.

- Wild sheep managers should coordinate with local weed or pest management districts, or other applicable agencies or organizations involved with weed or vegetation management, to preclude the use of domestic sheep or goats for noxious weed or vegetation control in areas where association with wild sheep is likely to occur. Agencies should provide educational information and offer assistance to such districts regarding disease risks associated with domestic sheep or goats. Specific guidelines (Pybus et al. 1994) have already been developed and implemented in British Columbia, and are available at: <http://www.for.gov.bc.ca/hfp/publications/00006/>.

- Specific protocols for sampling, testing prior to translocation, and responding to disease outbreaks should be developed and standardized to the extent practical across state and federal jurisdictions. Several capture and disease-testing protocols have been developed and are available to wild sheep managers (Foster 2004, UC-Davis 2007, WAFWA 2009). Protocols should be reviewed and updated as necessary by the WAFWA Wildlife Health Committee (WHC) and presented to WAFWA Directors for endorsement. Once endorsed, agencies should implement the protocols, and the WHC should lead an effort to further refine and ensure implementation of said protocols.

- Agencies should coordinate and pool resources to support the ongoing laboratory detection and interpretation of important diseases of wild sheep. Furthermore, wild sheep managers should support data sharing and development and use of standardized protocols (WAFWA 2009). Interagency communication between wildlife disease experts such as the WAFWA Wildlife Health Committee (WHC) should be encouraged to enhance strategies for monitoring, managing and improving health of wild sheep populations through cooperative efforts.

- Wild sheep management agencies should develop educational materials and outreach programs to identify and interpret the risk of association between wild sheep and domestic sheep or goats for producer groups, owners of small and large farm flocks, animals used for packing and 4-H animals. In some cases, regulation may be necessary to maintain separation.

RECOMMENDATIONS TO BLM, USFS, PARKS, PROTECTED AREAS AND OTHER APPLICABLE LAND MANAGEMENT AGENCIES

- Joint federal land management agency guidelines on management of domestic sheep or goats in wild sheep habitat should be developed and included in broad agency policy documents. Guidelines should be based on the need to minimize risk of association and provide effective separation between domestic sheep or goats and wild sheep.



Photo by: Todd Nordeen (NGPC)



Photo by: Debra Hamilton (CDFG)

Management Recommendations

Approved guidelines should not include an automatic “sunset” provision or expiration date but, if there is a maximum longevity (i.e., a “sunset clause”) specified by federal policy and if appropriate and timely review cannot be completed, guidelines should remain in effect, rather than becoming obsolete, until any mandated review can be completed.

■ The use of domestic sheep or goats as pack animals by persons that travel in identified wild sheep habitat should be prohibited by the appropriate management agency (e.g., USDA Forest Service 2011). Where legislation or regulations are not already in place, an outreach program to inform

potential users of the risks associated with that activity should be implemented to discourage use of domestic sheep or goats as pack animals.

■ Land management agencies that regulate or are responsible for domestic sheep or goat grazing allotments, trailing routes, vegetation management, use as pack stock, or any other uses involving domestic sheep or goats should only authorize such use(s) outside of occupied wild sheep range.

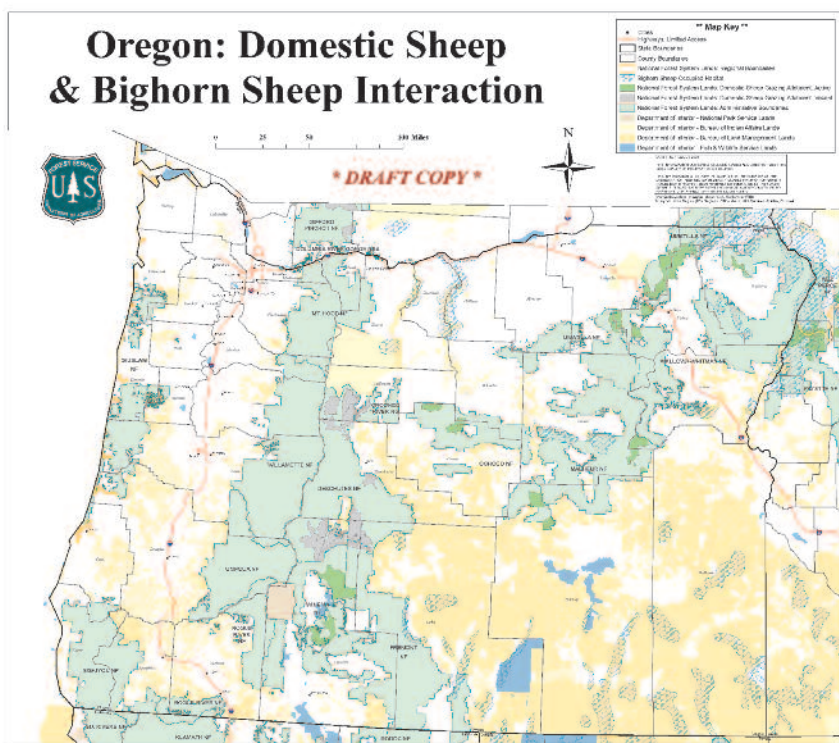
■ Land management agencies should require immediate notification by permittees and their herders of association between wild sheep and domestic sheep or goats and in no case should it be more than within 24 hours of any such event. Notification procedures, including phone numbers and contact information for permittees and use of satellite phones in backcountry settings, should be outlined in Annual Operating Instructions for grazing allotments and trailing permits, and should include consequences for failure to report.

■ Land management agencies should map active and inactive domestic sheep or goat grazing allotments and trailing routes, including information on dates of use and contact information for responsible grazing or trailing permittees.

■ Land management agencies must ensure that advance written instructions (such as USFS Annual Operating Instructions) exist, and that they address management, retrieval, and disposition of domestic sheep or goats present on public lands prior to or after permitted grazing or trailing dates.

■ Land management agencies should work collaboratively with state, provincial, and territorial wildlife and agricultural interests to develop written agreements that address management, retrieval, and disposition of domestic sheep or goats occupying public lands where there is no permitted use. Such agreements should also address the presence of feral sheep or goats and other exotic ungulates, especially ovines such as aoudad, red sheep, urial, or argali that are detected on public lands.

■ Land management agencies should review domestic sheep allotment boundaries or other use areas, such as trailing routes, and reconfigure boundaries or routes to avoid or minimize overlap with occupied wild sheep habitat. Techniques available to accomplish this include the use of geographic or topographic



Provided by: Chans O'Brien (USFS)



Management Recommendations

barriers that enhance species separation, and temporal or spatial separation resulting from implementation of novel domestic sheep or goat grazing management strategies.

- Land management agencies should undertake habitat enhancements that improve wild sheep habitat outside allotment boundaries in an effort to attract wild sheep away from domestic sheep allotments.

- Land management agencies should undertake water developments to divert wild sheep away from domestic sheep allotments or domestic sheep or goats away from areas used by wild sheep.

- Land management agencies should ensure that Annual Operating Instructions require careful management and vigilant herding to minimize potential association between wild sheep and stray domestic sheep or goats. A count-on, count-off inventory of domestic sheep or goats must be required as a condition of operation with follow-up provisions to account for missing livestock.

- In areas of high risk of association, trucking should be required to minimize risks associated with trailing. Trucking of domestic sheep or goats is preferred to trailing because there is less chance of straying and, thereby, less likelihood of association with wild sheep, particularly when domestic sheep are in estrus.

- Land management agencies should require marking of all permitted domestic sheep and goats to provide for rapid ownership identification of stray animals.

- In the event of trailing, on-site compliance monitoring to minimize strays must be conducted by the permittee or the land management agency.

- Land use or resource management plans should explicitly address the potential for domestic sheep or goats to associate with wild sheep. Land use plans should evaluate the suitability of permitting activities involving domestic sheep or goats, and determine the best course of action with respect to wild sheep conservation. Plans should also identify general areas of public land where domestic sheep or goats cannot be permitted for weed control, commercial grazing, recreational packing, vegetation management, or other uses.

- Land management agencies should coordinate with appropriate entities involved in weed control programs that use domestic sheep or goats on public or Crown lands (Pybus et al. 1994), adjoining private lands, or state, provincial, and territorial wildlife habitat management areas to minimize risk of association between domestic sheep or goats and wild sheep.

- Within occupied or suitable wild sheep habitat, where topography, vegetation, and other parameters allow, conversions of allotments from domestic sheep or goats to types of domestic livestock that pose a lower risk of disease transmission to wild sheep should be implemented.

- Within suitable, historic wild sheep habitat not currently occupied by wild sheep, agencies should not convert cattle grazing allotments to domestic sheep or goat grazing, or allow trailing if restoration of wild sheep populations is an agency goal.



Photo by: AZGFD



Photo by: Justin Binfet (WGFD)

Management Recommendations

■ Under emergency conditions, stocking of allotments not currently under permit to domestic sheep or goats should be permitted only after an adequate risk assessment has been completed. Any such assessment must include appropriate documentation and the conclusion that effective separation can be assured, and can be accomplished via project-level NEPA analysis.

■ Land management agencies should incorporate state, provincial, or territorial wild sheep management plans either in, or as supplements to, federal resource or land use management plans, and collaborate with wildlife agencies to ensure comprehensive risk assessments (Clifford et al. 2009, USDA Forest Service 2010a, b) of domestic sheep or goat grazing allotments or trailing routes in wild sheep habitat are thorough and complete. To accomplish this objective, training adequate to allow the preparation of such assessments must be provided.

Photo by: Mike Cox (NDOW)



■ Where mandatory buffer zones (frequently cited as a minimum of 9 airline miles [14.5 km]) between domestic sheep or goats and wild sheep have been used to minimize association, it should be recognized that buffer zones apply to herds or populations of wild sheep, rather than individual wandering wild sheep. In some cases, buffer zones have been effective in reducing association between wild sheep and domestic sheep or goats. However, in contiguous wild sheep habitat where movements by wild sheep have the potential to exceed *a priori* expectations, buffer zones may not be effective or practical (Schommer and Woolever 2001).

■ Topographic features or other natural or man-made barriers (e.g., fenced, interstate highways) can be effective in minimizing association between wild sheep and domestic sheep or goats. Site-specific risk assessments should be completed to evaluate the efficacy of using natural barriers, defined buffer zones, or other actions to minimize risk of contact. Given the wide range of circumstances that exists across jurisdictions, buffer zones may not be needed in all situations. Conversely, buffer zones should not be precluded as an effective method to address potential association between wild sheep and domestic sheep or goats.

■ Land management agencies, in collaboration with jurisdictional domestic sheep or goat health agencies, should work with producers and permittees to prevent turnout or use of sick or diseased domestic sheep or goats on grazing allotments and trailing routes. Sick or diseased domestic sheep or goats can increase risk of association with wild sheep because they likely are less able to keep up with their bands and are more prone to straying. Sick or diseased animals observed on the range should be reported to land management agency personnel immediately, and inter-agency coordination to address the situation should promptly occur. Further, responsible agencies must require that domestic sheep or goats are in good health before being turned out. For example, Alberta and British Columbia have developed health certification protocols (Pybus et al. 1994) that must be complied with before domestic sheep are turned out for vegetation management in conifer regeneration efforts (available at: <http://www.for.gov.bc.ca/hfp/publications/00006/>). We emphasize that the higher the risk of association between domestic sheep or goats with wild sheep, the higher the certainty of domestic animal health should be. Further, it must be recognized that even clinically healthy domestic sheep or goats can still carry pathogens that are transmissible to wild sheep, and thus, pose a significant risk to wild sheep.

Photo by: Todd Nordeen (NGPC)





Management Recommendations

■ Proportional to risk of association between domestic sheep or goats and wild sheep, land management agencies should work with stakeholders to implement a variety of management practices. Examples include: herders, dogs or other guarding animals trained to repel animals foreign to domestic sheep bands or goat flocks (wandering wild sheep or various predators), regular counts, removal of sick animals, confinement of domestic sheep or goats at night, adequate fencing configurations, covenants, allotment retirements, conversion of class of livestock, trucking versus trailing, and others. Effectiveness of management practices designed to reduce risk of association are not proven (Baumer et al. 2009, Schommer 2009) and therefore should not be solely relied upon to achieve effective separation. Such practices could however, help achieve separation when applied outside of occupied wild sheep range or connected and potentially mitigate impacts associated with straying domestic sheep or goats, or wandering wild sheep.

■ Land management agencies and wildlife agencies should cooperatively manage for quality wild sheep habitat and routinely monitor habitat to detect changes in condition.

■ In areas where association between wild sheep and domestic sheep or goats is likely, land management agencies should post advisory signs at trailheads, campgrounds, and other high-use areas that are designed to educate visitors about the issue of interaction and to encourage prompt reporting of association of wild sheep with domestic sheep or goats. Agencies should also ensure that individuals keep dogs under immediate voice control or on leash to prevent scattering of domestic sheep or goats in permitted areas, or disturbances to wild sheep.

■ Land management agencies should clearly define the processes, protocols, and timelines for short-term or emergency management actions when intervention is needed to minimize risk of association between wild sheep and domestic sheep or goats.

■ Land management agencies should develop programs to foster and recognize the benefits of compliance, cooperation, and cost-sharing in efforts to prevent commingling of wild sheep and domestic sheep or goats on shared ranges.

■ In collaboration with wild sheep management agencies, land management agencies should investigate and implement an option to allow the permittee or producer, or appropriate agency representatives, to remove commingling wild sheep and, where not already established, develop or clarify legal authority for removing stray domestic sheep from public lands by lethal means.

■ Risk assessment should be conducted on an appropriate geographic scale regardless of jurisdictional boundaries. Recognizing the limits of regulatory authority, land management agencies should consider private in-holdings and adjacent private lands when conducting risk assessments.

■ Land management agencies should closely evaluate timing of permitted domestic sheep or goat grazing or trailing activities to reduce risk of disease transmission. For example, grazing estrous domestic females heightens



Photo by: Robin Fehlau (BLM)



Photo by: Stephanie Steinhoff (CPW)

Management Recommendations

attraction and increases the probability of association between wild sheep and domestic sheep, and should be eliminated where benefits can be accrued.

■ In areas of high risk of association between wild sheep and domestic sheep or goats, agencies and permittees should ensure enhanced monitoring of grazing and trailing patterns using global positioning system (GPS) collars or other technology that provide detailed data on movements and grazing patterns. While enhanced monitoring will not reduce risk of association, it is vital for development of meaningful risk assessments and to ensure appropriate management recommendations are taken to achieve effective separation.



Photo by: Mike Gox (NDOW)



Photo by: Helen Schwantje (BC FLNRO)

RECOMMENDATIONS TO WILD SHEEP AND OTHER CONSERVATION ORGANIZATIONS

- Recognize and support efforts of wild sheep management agencies and industry leaders in maintaining effective separation.
- Assist wildlife and land management agencies with development of informational brochures and other materials that identify and explain risk of association between wild sheep and domestic sheep or goats.
- Assist wildlife and land management agencies with educational efforts regarding risks associated with the use of domestic sheep or goats as pack animals in wild sheep habitat. If use is authorized, encourage participants to closely control, tether, and night-pen their pack stock. Encourage prompt reporting of association between wild sheep and domestic sheep or goats, and promote a reporting system for monitoring association between wild sheep and domestic sheep or goats.
- Maintain or establish open lines of communication with domestic sheep or goat producers and industry organizations to reduce polarization. Jointly organized and cooperatively-funded workshops on risk assessment, identification of practical strategies to achieve effective separation, development and distribution of pamphlets or brochures, and public speaking opportunities are tangible examples of collaborative, multi-disciplinary approaches to address potential disease transmission.
- Continue to negotiate alternatives or incentives for domestic sheep or goat permittees to shift their operations to grazing allotments outside of wild sheep habitat. Advocate that permittees convert to a different class of livestock with lower risk of disease transmission or waive permitted domestic sheep or goat use in areas where risk assessment indicates high potential for association with wild sheep.

- Encourage and support development and funding of cooperative research, and encourage agencies and conservation groups to commit resources necessary to maintain wild sheep populations.

SUGGESTED MANAGEMENT PRACTICES FOR DOMESTIC SHEEP AND GOAT PERMITTEES

The following suggestions are based largely on recommendations provided by CAST (2008), Baumer et al. (2009), or USAHA (2009), and are intended to provide a responsible and common-sense approach for reducing risk of association. However, there is no science-based evidence or evaluation that assesses the effectiveness of these actions to reduce risk or enhance separation (Schommer 2009).



Management Recommendations

■ Implement the following reporting and record keeping procedures or use an existing standard such as the BC (Appendix B) or Wyoming (Appendix C) models:

- Require prompt, accurate reporting by herders working on domestic sheep or goat grazing allotments where association of wild sheep with domestic sheep or goats is possible.
- Support fluency in English or translators for foreign herders in order to facilitate accurate reporting.
- Require sheepherders to use cellular or satellite phones or two-way radios, and location equipment such as GPS receivers to report and record grazing movements and encounters with wild sheep. Seek cost-sharing partnerships for providing communications equipment when an operator changes grazing management practices for the sole purpose of minimizing domestic sheep association with wild sheep. Partnerships could include wildlife management agencies, federal land managers, or private organizations.
- Require herders to record GPS locations, counts, losses and other information in a log book.

■ Place only experienced, informed and responsible sheepherders on allotments located near wild sheep habitat.

■ Ensure that all domestics are individually marked and traceable to source flocks.

■ Conduct full counts when trailing, immediately any time scattering occurs and regularly during general grazing.

■ Develop agreements between permittees and wildlife agencies that provide for locating and reacquiring all stray domestic sheep, either dead or alive. In the event of missing domestic sheep, a comprehensive search should be initiated immediately and the land manager and state wildlife agency must be notified of missing and subsequent recovery of animals.

■ Develop a detection and response protocol that includes:

- Reporting of wild sheep and domestic sheep associations (animal counts and GPS location) to the appropriate wildlife agency.
- Reporting of stray or missing domestic sheep to the land management agency who will, in turn, report that information to the wildlife agency.
- Removal of stray domestic sheep by the permittee, land manager or wildlife agency personnel.
- Removal of individual commingling wild sheep by wildlife agency personnel.
- Collection of standardized diagnostic samples from stray domestic sheep or commingling wild sheep.

■ Utilize the following trailing procedures:

- Conduct full counts when moving on and off each allotment/grazing site.

- Truck domestic sheep through “driveway” areas that pass through occupied wild sheep habitat.
- Truck in water (if needed) to reduce straying.
- Immediately remove animals unable to stay with the flock/herd and move them to a base property.
- Avoid trailing more than 5 miles per day and stop trailing when sheep or lambs show signs of fatigue. Provide for a “babysitter” or removal of lagging sheep when trailing.
- In the event that all animals cannot be accounted for, the permittee must advise the responsible agency and initiate efforts to locate missing animals and implement removal protocol as necessary.

■ Sick domestic sheep should be removed from allotments immediately and must never be abandoned.



Management Recommendations

- Select herder's camp, nighttime bedding ground, and midday bedding ground locations that maintain communication between guard dogs and herding dogs by smell, sound (barking) and sight, and to take advantage of differences in the sleep cycles of guard dog and herding dogs. Place mature and effective guard dogs and herding dogs with domestic sheep (at least 2 of each per 1000 animals) and do not use female dogs in heat.

- If grazing on federal lands, comply with established "bed ground" standards. Where conditions permit, construct temporary electric or boundary fences to ensure that domestic sheep remain within selected bedding grounds.

SUGGESTED MANAGEMENT PRACTICES ON PRIVATE LANDS

- Recognize that domestic sheep or goat farming on private lands can influence wild sheep population viability on adjacent public or other private lands.

- Report any observed association between wild sheep and domestic sheep or goats on or near private land to the appropriate wildlife conservation agency.

- Cooperate with wildlife agencies in reporting and removing feral sheep or goats and other exotic bovine ungulates such as aoudad, red sheep, urial, or argali that are detected within or near wild sheep habitat.

- Participate in cooperative educational efforts to enhance understanding of the issues of disease transmission between domestic sheep or goats and wild sheep.

- Do not release or leave unattended domestic sheep or goats in areas where they may seek, or be sought, by wild sheep.

- Cooperate with appropriate agencies, agricultural and producer associations, conservation organizations, and other interested stakeholders to develop effective, comprehensive risk management approaches to help ensure effective separation between wild sheep and domestic sheep or goats, consistent with private property rights in and near wild sheep habitat.

- Possible approaches include, but are not limited to, changing species or class of livestock, purchase of land or the domestic sheep or goats, use of methods to ensure physical separation, or development of conservation incentives, bylaws, covenants, or legislation.

- Consider partnerships with non-governmental organizations and wild sheep advocate groups for cost sharing on risk management/mitigation strategies such

as fencing, or other domestic sheep or goat management actions that reduce risk of disease transmission from private flocks to wild sheep.

- Support "effective separation" fencing standards that are designed to prevent nose-to-nose contact and aerosol transmission through adequate physical distance, in order to reduce transmission of respiratory disease agents. Examples include: electric outrigger fences (2 feet from page (woven) wire fencing) and double fencing (two page-wire fences with a minimum spacing of at least 10 feet). A combination of fencing methods with or without the use of effective livestock guardian dogs may be most effective to ensure that wild sheep do not physically contact domestic sheep or goats on private land.

- Participate in or support cooperative research to enhance understanding and test mitigation protocols for disease risk management.

- Carefully consider the consequences of using domestic sheep or goats for weed control on private lands where association with wild sheep could occur. Work with agencies to develop alternative weed management strategies to reduce risk of association, while adequately managing weed problems.

Photo by: David Wetzel (Texas Bighorn Society)



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Glossary of Terms

Allotment: A portion of a landscape where livestock grazing of a plant community is prescribed according to a specific land use plan or legally defined regulatory authority.

Annual Operating Instructions: Specific language included in a term grazing or trailing permit file; reviewed each year with the permittee, prior to turnout of livestock on a grazing allotment or trailing route.

Association: Close proximity between wild sheep and domestic sheep or goats, potentially leading to direct physical contact and potential disease transmission.

Augment: To intentionally introduce wild sheep from one or more source populations into another existing wild sheep population, to enhance the recipient population demographically or genetically.

Buffer zone: A defined and delineated space on a landscape established by wildlife managers to reduce association and the potential for disease transmission between wild and domestic sheep or goats across that geographic space.

Bighorn sheep: A member of the species *Ovis canadensis* found throughout the mountains of western North America from the Peace River in Canada to northern Mexico and east to the Badlands of the Dakotas.

Contact: Direct contact between body parts of two animals during which a disease might be transmitted from one to another. In this document, “contact” typically refers to nose-to-nose or face-to-face interactions that may lead to the transmission of respiratory disease via secretions or aerosols. Synonymous with “Interaction.”

Connectivity: Creating or maintaining networks of habitat that connect fragmented habitats, thus linking population segments of wildlife. Connectivity allows gene flow and enhances long-term species survival.

Conservation Incentives: In direct contrast to regulation-based conservation, incentive-based conservation provides economic, management or esthetic benefits to individuals or corporations to encourage them to conduct management activities that have positive conservation consequence to wildlife or wildlife habitat. Examples are: private land conservation easements, direct lease agreements for grazing rights for conservation purposes, or a trade/exchange of equal value grazing rights among various partners to minimize wildlife-domestic livestock conflict.

Die-off: A large-scale mortality event that impacts many animals from a population and may have significant demographic consequence for the long-term persistence of that population. In this report, such mortality events are usually caused by respiratory disease epidemics involving bacterial or other pathogens alone or in various combinations.

Disease: The word disease means literally “free of ease.” Disease is any impairment that modifies or interferes with normal functions of an animal, including responses to environmental factors such as nutrition, toxicants, and climate. Typically, disease involves transmission of, and exposure to, some infectious agent but it may involve non-infectious causes such as congenital defects.

Dispersal: The process whereby individuals leave one habitat or landscape to seek another habitat or landscape in which to live.

Double fencing: Two fences running parallel around a landscape or pasture to prevent contact between animals across the fence line, designed to inhibit disease transmission.

Effective separation: Spatial or temporal separation between wild sheep and domestic sheep or goats, resulting in minimal risk of contact and subsequent transmission of respiratory disease between animal groups.

Feral: An animal of a domestic species that resides in a non-domestic setting and is not presently owned or controlled.

Historic habitat: Based on historic records, landscape that was previously occupied by bighorn sheep and thought to have provided necessary requirements to sustain a wild sheep population through time.

Interaction: Direct contact between body parts of two animals during which a pathogen might be transmitted from one to another. In this document, “interaction” typically refers to nose-to-nose or face-to-face interaction that may lead to the transmission of respiratory disease via secretions or aerosols. Synonymous with “Contact”.

Metapopulation: An assemblage of populations, or a system of local populations (demes) connected by movement of individuals (dispersal) among various population segments.

Movement corridor: Routes that facilitate movement of animals between habitat fragments.



Appendix A

Occupied habitat/range: Suitable habitat in which a wild sheep population currently exists.

Preferred: A specific management action that *should* be chosen over another, whenever possible:

Radio collars: Transmitters fitted on neckband material to monitor animal locations.

Global Positioning System (GPS): A radio transmitter fitted on neckband material linked with orbiting satellites; animal locations can be precisely triangulated from space, with the location data then electronically stored in a memory chip or transmitted by various methods for data retrieval.

Very High Frequency (VHF): A radio transmitter fitted to neckband material transmitting in the Very High Frequency range that can be located from the ground or aircraft using a telemetry receiver.

Removal: Physical extraction of domestic sheep or goats, or wild sheep, to eliminate (permanently or temporarily) occupancy of that range or habitat.

Risk/Risk Assessment/Risk Management: In this context, evaluation of the probability that a wild sheep population could experience a disease event with subsequent demographic impacts. Identification of what factors might contribute to the probability of a disease event. Management actions taken to reduce the probability of exposure and/or infection among or between animals. Examples of risk management include separation of infected and non-infected animals, treatment of infected individuals, vaccination, manipulations of the host environment, or manipulations of the host population.

- Qualitative Risk Assessment: Interpretation and analysis of factors that cannot necessarily be measured.
- Quantitative Risk Assessment: Use of tangible data and measurements.

Spatial separation: A defined physical distance between animal populations.

Stray: A domestic sheep or goat physically separated from its flock or band.

Stressor: A specific action or condition that causes an animal to experience stress and the subsequent physiological results of that stress.

Suitable habitat: Landscape that has all necessary habitat requirements to sustain a wild sheep population through time.

Temporal separation: Segregating animal populations over time to prevent association, such that they may occupy the same physical space but at different times.

Thinhorn sheep: A member of the species *Ovis dalli* occurring in Alaska, Yukon Territory, Northwest Territories, and northern British Columbia.

Transmission: The physical transfer (direct or indirect mechanisms) of a disease agent from one animal to another, either within an animal population or between animal populations. In some instances, transmission can lead to full expression of disease in individuals or populations.

Transplant: An intentional movement of wild sheep from a source population to other suitable wild sheep habitat, either currently occupied or not. (Also called “translocation” in some documents.)

Trailing: The planned ambulatory movement of domestic sheep or goats across a landscape or within a corridor to reach a destination where grazing or use will be allowed.

Unoccupied habitat/range: Suitable habitat in which a wild sheep population does not currently exist.

Viability: The demographic and genetic status of an animal population whereby long-term persistence is likely.

Wandering Wild Sheep: Wild sheep, primarily but not always young, sexually-mature rams, occasionally traveling outside of normally anticipated or expected wild sheep range and adjacent habitat. Removal of wandering wild sheep typically does not have population-level implications for wild sheep. Conversely, failure to respond to wandering wild sheep may result in significant, adverse population-level impacts.

British Columbia Domestic-Wild Sheep Separation Project Contact Protocol

The following protocols outline *the steps to be taken when reports of wild sheep contact with domestic sheep are received by the Ministry of Environment* in one of several ways:

1. Regular report from public to regional office (Conservation Officer Service or Wildlife Section):

- Contact reported to Regional office.
- Assessment of situation by sheep biologist and COS, in consultation with wildlife veterinarian
- If close contact is confirmed and is considered a high risk situation, consider the following options:
 - a. Kill bighorn and save carcass – sample bighorn and/or domestics in consultation with wildlife veterinarian
 - b. Continue to monitor bighorn herd in area – observe and record general signs of health
 - c. Do nothing – but keep records
- If contact is unsubstantiated/considered low risk, continue to monitor bighorn herd in area, alert and encourage mitigation measures with domestic producers in area to ensure separation.

2. Regular report from public to Call Line.

- Contact reported to Call Line; Call Line staff forwards to regional COS.
- Assessment of situation by COS and sheep biologist, in consultation with wildlife veterinarian
- If close contact is confirmed and is considered a high risk situation, consider the following options:
 - a. Kill bighorn and save carcass – sample bighorn and/or domestics in consultation with wildlife veterinarian
 - b. Continue to monitor bighorn herd in area – observe and record general signs of health
 - c. Do nothing – but keep records
- If contact is unsubstantiated/considered low risk, continue to monitor bighorn herd in area, alert and encourage mitigation measures with domestic producers in area to ensure separation.

3. Out of hours call from public to Call Line.

- Contact reported to Call Line; Call Line staff forwards to regional COS officer-on-call.
- Assessment of situation by COS officer-on-call – contacts sheep biologist and wildlife veterinarian, if possible for consultation
- If sheep biologist and wildlife veterinarian cannot be contacted, biologist and veterinarian will support COS decision and action. COS will inform sheep biologist and wildlife veterinarian by email of the situation and action taken.
- If close contact is confirmed and is considered a high risk situation, consider the following options:
 - a. Kill bighorn and save carcass – sample bighorn and/or domestics in consultation with wildlife veterinarian
 - b. Continue to monitor bighorn herd in area – observe and record general signs of health
 - c. Do nothing – but keep records
- If contact is unsubstantiated/considered low risk, continue to monitor bighorn herd in area, alert and encourage mitigation measures with domestic producers in area to ensure separation.

Appendix C



WYOMING GAME AND FISH DEPARTMENT

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MEMORANDUM

TO: Wildlife Division Employees

FROM: Jay Lawson, Chief, Wildlife Division

COPY TO: Terry Cleveland, Gregg Arthur, File

SUBJECT: **PROTOCOL FOR HANDLING THE COMMINGLING
OF BIGHORN SHEEP AND DOMESTIC SHEEP/GOATS**

Due to the threat of disease transmission and subsequent bighorn sheep die-offs, the following protocol should be followed.

Wandering Bighorn Sheep:

Where there is known, suspected, or likely contact by a wandering bighorn sheep with domestic sheep/goats:

- If possible, that bighorn(s) should be live-captured and transported (one-way) to our Sybille Research Unit.
- If that bighorn(s) cannot be live-captured, that bighorn(s) should be lethally removed (per authority of Chapter 56) and, if possible, transported (either whole or samples) to our Sybille Unit or our WGFD Lab in Laramie.

Stray Domestic Sheep/Goat:

Where there is known, suspected, or likely contact by a stray domestic sheep/goat with bighorn sheep:

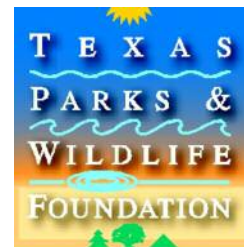
- The owner of such livestock should be notified and asked to remove the stray sheep/goat to eliminate the threat of disease transmission; however, it will be the owner's prerogative to determine what course of action should be taken.

Reporting:

All documented commingling and any actions taken must be reported to the employee's immediate supervisor, Wildlife Administration as well as the Bighorn Sheep Working Group Chairman, presently Kevin Hurley.

"Conserving Wildlife - Serving People"

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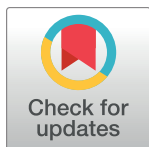
RESEARCH ARTICLE

Exposure of bighorn sheep to domestic goats colonized with *Mycoplasma ovipneumoniae* induces sub-lethal pneumonia

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Abstract

Background

Bronchopneumonia is a population limiting disease of bighorn sheep (*Ovis canadensis*) that has been associated with contact with domestic Caprinae. The disease is polymicrobial but is initiated by *Mycoplasma ovipneumoniae*, which is commonly carried by both domestic sheep (*O. aries*) and goats (*Capra aegagrus hircus*). However, while previous bighorn sheep comingling studies with domestic sheep have resulted in nearly 100% pneumonia mortality, only sporadic occurrence of fatal pneumonia was reported from previous comingling studies with domestic goats. Here, we evaluated the ability of domestic goats of defined *M. ovipneumoniae* carriage status to induce pneumonia in comingled bighorn sheep.

Methodology/Principal findings

In experiment 1, three bighorn sheep naïve to *M. ovipneumoniae* developed non-fatal respiratory disease (coughing, nasal discharge) following comingling with three naturally *M. ovipneumoniae*-colonized domestic goats. Gross and histological lesions of pneumonia, limited to small areas on the ventral and lateral edges of the anterior and middle lung lobes, were observed at necropsies conducted at the end of the experiment. A control group of three bighorn sheep from the same source housed in isolation during experiment 1 remained free of observed respiratory disease. In experiment 2, three bighorn sheep remained free of observed respiratory disease while comingled with three *M. ovipneumoniae*-free domestic goats. In experiment 3, introduction of a domestic goat-origin strain of *M. ovipneumoniae* to the same comingled goats and bighorn sheep used in experiment 2 resulted in clinical signs of respiratory disease (coughing, nasal discharge) in both host species. At the end of experiment 3, gross and histological evidence of pneumonia similar to that observed in experiment 1 bighorn sheep was observed in both affected bighorn sheep and domestic goats.

Conclusions/Significance

M. ovipneumoniae strains carried by domestic goats were transmitted to comingled bighorn sheep, triggering development of pneumonia. However, the severity of the disease was

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markedly milder than that seen in similar experiments with domestic sheep strains of the bacterium.

Introduction

Historical and contemporary field observations suggest that contacts with domestic sheep are followed by pneumonia outbreaks in previously healthy bighorn sheep populations [1–3]. Supporting these observations, >95% of 90 bighorn sheep in eleven studies involving contact with domestic sheep suffered fatal pneumonia within 100 days, as reviewed in [4]. However, the potential role of domestic goats in transmitting pneumonia pathogens to bighorn sheep is less clear, with relatively fewer observed bighorn sheep pneumonia outbreaks following domestic goat contacts [5–7] and with relatively little fatal bighorn sheep pneumonia observed in two experimental comingling studies with domestic goats (22% of 9 bighorn sheep) [5, 8]. Domestic goats may transmit respiratory pathogens when they contact bighorn sheep on or near bighorn sheep home ranges as stray animals from farmsteads, or when used for weed control, or when commercial operations are grazed on public lands in or near bighorn sheep home ranges, or when used as pack animals supporting back-country recreation. In addition, bighorn sheep may encounter domestic goats on private lands within or adjacent to bighorn ranges. Therefore, it is important to understand the risk that contact with domestic goats may pose for bighorn sheep health.

Mycoplasma ovipneumoniae, proposed as the primary pathogen that triggers bighorn sheep pneumonia outbreaks [9, 10] followed by persistently impaired recruitment resulting from recurrent lamb pneumonia [11, 12], has a host range limited to Caprinae [13]. Distinct host-specific clades of *M. ovipneumoniae* have recently been reported in domestic sheep and goats [7, 14]. In a Washington state survey of goat farms adjacent to bighorn sheep habitat, this pathogen was carried asymptotically by animals on 7 of 16 goat farms, and by 58% of individual goats on positive farms [15].

Like bighorn sheep pneumonia, respiratory disease of domestic sheep and goats is polymicrobial, frequently including *M. ovipneumoniae* (and perhaps also *Mycoplasma arginini*), *Mannheimia haemolytica*, *Pasteurella multocida*, and other pathogens. Chronic and persistent coughing is the main clinical sign observed associated with *M. ovipneumoniae* infection of domestic lambs, which is often described as summer pneumonia in Australia and New Zealand, or as coughing syndrome in the United States [13, 16]. Clinical severity ranges from mild illness associated with reduced growth rates to fatal polymicrobial pneumonia [13, 17]. *M. ovipneumoniae* has also been associated with outbreaks of severe respiratory disease in goats [13, 18, 19].

The *M. ovipneumoniae* carriage status of the animals used in previously published experimental domestic goat-bighorn sheep comingling studies was not defined [5, 8] and so it is possible that the low death rate reported in those studies was due to the absence of *M. ovipneumoniae* in the comingled goats. Here we tested the hypothesis that exposure to domestic goats carrying *M. ovipneumoniae* results in epizootic pneumonia in bighorn sheep by conducting a series of experimental comingling studies with domestic goats of defined *M. ovipneumoniae* carriage status.

Materials and methods

Ethics statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and in conformance with

United States Department of Agriculture animal research guidelines, under protocols #03793 and #04482 approved by the Washington State University Institutional Animal Care and Use Committee. As described in those protocols, euthanasia was to be performed by intravenous injection of sodium pentobarbital for animals observed to be in severe distress associated with pneumonia during the study and prior to any necropsy examination for surviving animals at the end of each experiment.

Experimental animals

Six healthy, *M. ovipneumoniae*-unexposed, pregnant adult bighorn ewes were provided by the Washington Department of Fish and Wildlife on January 25, 2014 from the Clemans Mountain population for these studies. This population had previously been confirmed to be uninfected and unexposed to *M. ovipneumoniae* as determined by repeated testing with both competitive enzyme-linked immunosorbent assay (cELISA) serology and nasal swab polymerase chain reaction (PCR) testing [20]. Blood and nasal swab samples collected from the six experimental animals on the day of capture were also negative by *M. ovipneumoniae* cELISA and PCR tests. The ewes were randomly allocated to two experimental groups of three animals each. Sampling or moving the bighorn sheep was conducted under anesthesia (butorphanol tartrate, azaperone tartrate, and medetomidine hydrochloride, reversed with atipamezole and naltrexone, BAM kit, Wildlife Pharmaceuticals, Inc. Windsor CO). Six domestic goats were purchased from three local private owners for use in the experiments. Domestic goats used in experiment 1 (group 1) included a 5+ year Saanen wether previously used as a pack animal, a 1 year old mixed breed wether intended for slaughter, and a 1 year old Boer wether from a herd used for weed and brush control. All were confirmed nasal carriers of *M. ovipneumoniae* based on PCR testing of nasal swab samples on at least two occasions prior to the study. Domestic goats used in experiments 2 and 3 (group 2) included one yearling Boer female and two yearling LaMancha wethers, all negative for *M. ovipneumoniae* by nasal swab PCR when purchased. Because they originated in a *M. ovipneumoniae*-positive herd, group 2 domestic goats were placed in isolation from other small ruminants and were serially re-tested for *M. ovipneumoniae* nasal carriage (5 times over a 4 week period) to confirm their negative status prior to the start of experiment 2.

Experimental design

Three experiments were conducted in which domestic goats (DG) of known *M. ovipneumoniae* status were comingled with susceptible bighorn sheep (BHS). In each experiment, comingled animals shared feed and water sources (pelleted hay supplemented with a corn/oats/barley mixture, free choice trace mineral salt, and a single frost-protected water source in each pen). Single roofed shelters were available in each pen, and plywood sheets covered the pen fences at the corners to provide windbreaks. All animals were observed daily.

Experiment 1. BHS group 1 (BHS29, BHS32, and BHS33; *M. ovipneumoniae*-negative) was comingled in a 372 m² pen (pen 1) with group 1 DG (DG1, DG2, and DG3; *M. ovipneumoniae*-positive) beginning February 18, 2014. During experiment 1, BHS group 2 (BHS27, BHS28, and BHS31; *M. ovipneumoniae*-negative) were housed in a separate 232 m² pen (pen 2) isolated >30 m from any other domestic or wild sheep or goats. All animals were observed closely for signs of respiratory disease for a 100 day period. On May 30, 2014, BHS group 1 animals were euthanized and subjected to complete necropsy examinations. DG group 1, having shown no clinical signs of respiratory disease during the experiment, were donated to the Washington State University veterinary teaching program.

Experiment 2. After group 1 animals were removed at the end of experiment 1, pen 1 was left empty for 40 days before BHS group 2 (*M. ovipneumoniae*-negative) was added on July 7,

2014. BHS group 2 composition was modified by the loss of BHS27 and by the addition of BHS28L (a lamb born to BHS28 on April 18, 2014 during the first experiment. On September 10, 2014, 65 days after BHS group 2 were placed in pen 1, DG group 2 (DG4, DG5, and DG6; *M. ovipneumoniae*-negative) was comingled to begin experiment 2, in which the comingled animals were again closely observed for signs of respiratory disease for a 100 day period.

Experiment 3. In experiment 3, a strain of *M. ovipneumoniae* obtained by nasal wash from a domestic goat (originating on a different farm than the source farms of DG groups 1 and 2) was introduced into the comingled BHS and DG from experiment 2, as follows: DG4 and DG5 were removed to an isolation pen where they were each inoculated with *M. ovipneumoniae* by instillation of 10 ml of ceftiofur-treated nasal wash fluids into conjunctivae and nasal passages. After confirmation of colonization by positive nasal swab PCR tests on days 3 and 7 following inoculation, they were re-commingled in pen 1 with *M. ovipneumoniae*-negative DG6 and BHS group 2 on February 16, 2015. The commingled animals were observed closely for signs of respiratory disease for a 100 day period. At the end of experiment 3, both BHS group 2 and DG group 2 were euthanized and subjected to complete necropsy examinations, as all had demonstrated signs of respiratory disease during the experiment.

Biosecurity

The study pens used for these experiments had not housed any BHS, DG or domestic sheep for >12 months prior to initiation of these experiments. Routine biosecurity measures during the experiments included broad physical separation (>1 km) and entirely separate animal care staff for pens 1 and 2. Animal care staff and researchers utilized personal clean protective equipment (coveralls, boots, and gloves) when entering study pens. Research staff occasionally entered both pens 1 and 2 on the same day, but if so always entered the *M. ovipneumoniae*-free pen 2 first, and changed personal protective equipment before entering pen 1.

Clinical scores

Clinical signs of respiratory disease were scored approximately 3 times per week using a point system: anorexia (1 point), nasal discharge (1 point), cough (2 points), nose licking (1 point), head shaking (1 point), ear paresis (1 point) and weakness/incoordination (1 point), as we have done previously [21].

Microbiological testing

Routine microbiological testing performed by the Washington Animal Diagnostic Laboratory (WADDL, accredited by the American Association of Veterinary Laboratory Diagnosticians), included detection of *M. ovipneumoniae*-specific antibodies in serum samples using competitive enzyme-linked immunosorbent assays (cELISA) [9, 22], detection of *M. ovipneumoniae*-specific DNA sequences by polymerase chain reaction (PCR) testing of nasal or bronchial swab eluates, culture-enriched swab samples (mycoplasma broth, 72 hrs, 35 C), or lung tissue samples [23, 24], detection of Pasteurellaceae in pharyngeal swab samples by conventional aerobic bacteriologic cultures and MALDI-TOF (Biotyper, Bruker, Woodlands TX) [25], and detection of neutralizing antibodies in serum samples indicating exposure to parainfluenza-3 and respiratory syncytial viruses by virus neutralization assays. PCR tests specific for detection of *M. haemolytica*, *B. trehalosi*, and *P. multocida*, and *lktA* (the gene encoding the principal virulence factor of *M. haemolytica* and *B. trehalosi*) were applied to DNA extracted from pneumonic lung tissues using previously described methods [26]. The 16S–23S ribosomal operon intergenic spacer (IGS) regions of *M. ovipneumoniae* recovered from animals in these studies

were PCR amplified and their DNA sequences determined in order to track transmission of specific *M. ovipneumoniae* strains (GenBank accession numbers pending) [7, 27].

Results

Experiment 1

All animals appeared clinically normal at the start of experiment 1. All BHS were negative for carriage (PCR testing of nasal swabs) and exposure to *M. ovipneumoniae* (cELISA testing of serum samples) (Table 1). DG were confirmed as carriers of *M. ovipneumoniae* based on detection of the bacterium by PCR on repeated nasal swab samples. Diverse pharyngeal Pasteurellaceae were detected in both BHS and DG during experiment 1 (Table 1). Group 1 BHS (BHS29, BHS32, and BHS33) and group 1 DG (DG1, DG2, and DG3) were commingled in pen 1 to begin the experiment, while group 2 BHS (BHS27, BHS28, and BHS31) were maintained in isolation in pen 2. Beginning 2–3 weeks after commingling, all group 1 BHS began showing signs of respiratory disease, including increased nasal discharge and coughing (Fig 1, S1 Table). Clinical signs of middle ear involvement (head shaking, ear droop) were rarely seen. After 70 days of illness, group 1 BHS exhibited decreasing clinical signs of respiratory tract disease, and group 1 DG remained clinically normal throughout the experiment. Group 1 BHS were euthanized and necropsied after 100 days of commingling. Uncommingled group 2 BHS remained healthy in pen 2 throughout experiment 1. At the end of experiment 1, the *M. ovipneumoniae*-negative status of group 2 BHS was re-confirmed by PCR and cELISA serology. BHS27 subsequently died from aspiration pneumonia, the onset of which followed the anesthesia conducted to permit sampling after experiment 1; at necropsy, BHS27 was also PCR- and ELISA-negative for *M. ovipneumoniae*.

All BHS ewes lambd during experiment 1. All lambs born to group 1 (goat-commingled, *M. ovipneumoniae*-positive) BHS died at less than 7 days of age, with signs of ocular, systemic and/or gastrointestinal tract disease (BHS29L, BHS32L, and BHS33L; Table 2). At necropsy,

Table 1. Microbiological status and pathologic lesions of animals in experiment 1.

Animal	Pen	Stage	Gross necropsy ¹	<i>M. ovipneumoniae</i> ²		<i>Pasteurellaceae</i> (<i>lktA</i>) ³
				PCR	cELISA (%I)	
BHS29	1	Pre	-	NotDet	NotDet (-12%)	P: Btre, Mhae+
		Post	LC (15% r; 5% l), CSE	Det: B, CS	Det (75%)	L: Pmul
BHS32	1	Pre	-	NotDet	NotDet (-13%)	P: Btre+
		Post	LC (5% l), CSE	Det: N, L	Det (60%)	L: NotDet
BHS33	1	Pre	-	NotDet	NotDet (3%)	P: Mhae+
		Post	LC (1% r) PA @ CSE	Det: B, N	Det (82%)	L: Psp
DG1	1	Both	-	Det: N	-	P: Btre, Mhae+
DG2	1	Both	-	Det: N	-	P: Mhae+
DG3	1	Both	-	Det: N	-	P: Btre, Mhae+
BHS27	2	Both	-	NotDet	NotDet (-7–3%)	P: Btre, Mhae, Psp
BHS28	2	Both	-	NotDet	NotDet (13–26%)	P: Btre, Mhae+, Pmul, Psp
BHS31	2	Both	-	NotDet	NotDet (7–16%)	P: Btre, Psp

¹ - = not done; LC = lung consolidation; CSE = cornual sinus exudate; PA = pleural adhesions; l = left lung; r = right lung.

²%I = Percent inhibition; NotDet = not detected; Det = detected; N = nasal passage swab; B = bronchus swab; CS = cornual sinus swab; L = lung tissue; — = not done.

³ Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; Btre = *Bibersteinia trehalosi*; Mhae = *Mannheimia haemolytica*; Pmul = *Pasteurella multocida*; Psp = *Pasteurella* species; + = *lktA* PCR positive; NotDet = No Pasteurellaceae detected.

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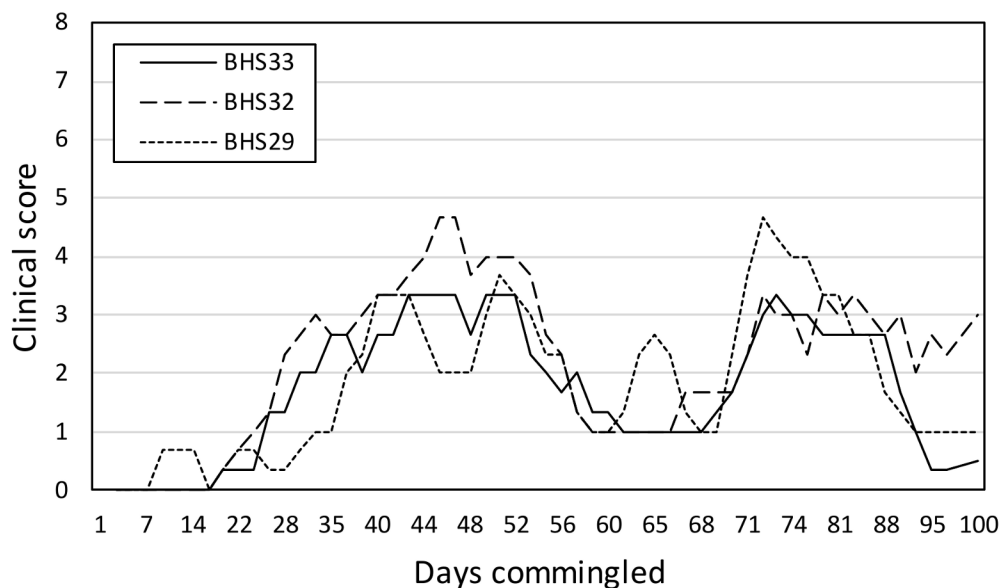


Fig 1. Clinical scores exhibited by bighorn sheep commingled with *M. ovipneumoniae* carrier domestic goats in Experiment 1. Three-day rolling average summed clinical scores based on observed anorexia, nasal discharge, cough, dyspnea, head shaking, ear paresis and weakness/incoordination. *M. ovipneumoniae* colonized DG were comingled with naïve BHS on day 0.

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none of these lambs had detectable pneumonia either grossly or histologically. The twin lambs born to group 2 (non-commingled; *M. ovipneumoniae*-negative) ewe BHS31 (BHS31L1 and BHS31L2) also died at 4 and 7 days of age, respectively, with signs of ocular, systemic and/or enteric disease. The lamb of group 2 BHS27 (BHS27L) was euthanized at 45 days of age necessitated by intractable diarrheal disease, while the lamb of BHS28 (BHS28L) survived.

Experiment 2

Group 2 DG, *M. ovipneumoniae*-free but carrying diverse pharyngeal Pasteurellaceae (Table 3), were comingled with group 2 BHS in pen #1. No respiratory disease signs were observed in either DG or BHS for >120 days during experiment 2.

Table 2. Outcomes and microbiological findings of lambs born during experiment 1.

Animal ID	Pen	Outcome ¹	<i>M. ovipneumoniae</i> ²		<i>Pasteurellaceae</i> (<i>IktA</i>) ³
			PCR	cELISA (% I)	
BHS29L	1	C (d6), death (d7)	Det: L N C	NotDet (0%)	P, L: Btre; C: Mhae+
BHS32L	1	C, D, death (d5)	Det: N	Det (50%)	P: Mhae+; C: Mhae+;
BHS33L	1	C, death (d7)	Det: N, B	Ind (49%)	P: Msp, Pmul; C: Mhae+; L: NotDet
BHS27L	2	D, euthanized d45)	NotDet	NotDet (2%)	NotDet
BHS28L	2	D (d54), survived	NotDet	NotDet (<4%)	P: Btre, Msp
BHS31L1	2	Death (d4)	NotDet	Not done	L: Mhae
BHS31L2	2	D, death (d10)	NotDet	NotDet (-9%)	L, C: Mhae+

¹C = conjunctivitis, D = diarrhea.

²%I = Percent inhibition; Det = detected; NotDet = not detected; Ind = indeterminate result; L = lung tissue; N = nasal passage swab; C = Conjunctival swab; B = Bronchial swab

³Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; C = conjunctival swab; Btre = *Bibersteinia trehalosi*; Mhae = *Mannheimia haemolytica*; Pmul = *Pasteurella multocida*; Msp = *Mannheimia* species; + = *IktA* PCR positive; NotDet = No Pasteurellaceae detected.

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Table 3. Microbiological status and pathologic lesions of animals in experiments 2 and 3.

Animal ID	Experiment	Gross necropsy ¹	<i>M. ovipneumoniae</i> ²		<i>Pasteurellaceae (IktA)</i> ³
			PCR ²	cELISA (%I)	
BHS28	Pre 2	-	NotDet	NotDet (26%)	P: Btre, Mhae+, Psp
	Post 2 Pre 3	-	NotDet	NotDet (12%)	P: Btre, Msp, Psp
	Post 3	PA (r, l)	Det: N, B	Det (79%)	P: Btre; L: Btre, Pmul
BHS28L	Pre 2	-	NotDet	NotDet (4%)	P: Btre, Mhae+, Psp
	Post 2 Pre 3	-	NotDet	NotDet (15%)	P: Msp
	Post 3	LC (5% r, 1% l)	Det: N	Det (83%)	P: Btre; L: Btre
BHS31	Pre 2	-	NotDet	NotDet (7%)	P: Btre, Psp
	Post 2 Pre 3	-	NotDet	NotDet (16%)	P: NotDet
	Post 3	No lesions seen	Det: N, B	Det (77%)	P: Btre; L: Btre, Psp
DG4	Pre 2	-	NotDet (x5)	NotDet (29%)	P: Btre, Mhae+
	Post 2 Pre 3	-	NotDet	NotDet (20%)	P: Btre
	Post 3	LC (10% r, 5% l)	Det: N, B	Det (79%)	L: Mhae
DG5	Pre 2	-	NotDet (x5)	NotDet (21%)	P: Btre, Psp+
	Post 2 Pre 3	-	NotDet	NotDet (21%)	P: Mhae
	Post 3	LC (5% r, 5% l)	Det: N, B	Det (55%)	P: Btre; L: Mhae, Btre
DG6	Pre 2	-	NotDet (x5)	NotDet (28%)	P: Btre; Mhae+, Psp+
	Post 2 Pre 3	-	NotDet	NotDet (27%)	P: Btre, Mhae
	Post 3	No lesions seen	Det: N, B	Det (63%)	P: Btre; L: Btre, Mhae

¹ - = not done, PA = pleural adhesions, LC = lung consolidation; l = left lung; r = right lung.

²%I = Percent inhibition; NotDet = not detected, Det = detected, N = nasal passage swab; B = bronchial swab.

³ Detection of Pasteurellaceae by either bacteriologic culture or PCR; P = pharyngeal swab; L = lung tissue; Btre = *Bibersteinia trehalosi*; Mhae = *Mannheimia haemolytica*; Pmul = *Pasteurella multocida*; Msp = *Mannheimia* species; Psp = *Pasteurella* species; + = *IktA* PCR positive; NotDet = No Pasteurellaceae detected by aerobic bacterial culture or by PCR.

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Experiment 3

Previously *M. ovipneumoniae*-free DG4 and DG5 began showing respiratory disease signs while still in isolation following *M. ovipneumoniae* inoculation and these signs continued after comingling in pen 1. All other pen 1 animals also developed signs of respiratory tract disease beginning shortly after re-comingling of DG4 and DG5 (Fig 2, S1 Table). The clinical respiratory disease scores of all animals in experiment 3 began diminishing after approximately 70 days of comingling. All animals were humanely euthanized for complete necropsy examinations at the end of experiment 3.

Necropsy findings

Gross necropsy examination of the bighorn sheep in experiments 1 and 3 and in the domestic goats in experiment 3 revealed limited areas of lung consolidation in most but not all animals (Tables 1 and 3). These areas were localized to the ventral tips or edges of the middle and anterior lobes, and were darker red in color and firmer on palpation than the adjacent normal lung tissues. In addition, several animals had strong fibrous adhesions between these same lung regions and the pericardial or parietal pleura, presumably representing resolved pleuritis. All animals in the study had similar histopathologic lesions that varied in severity, consisting of inflammation centered around bronchi and bronchioles and extending to include adjacent alveoli (Fig 3). Inflammation was characterized by peribronchiolar and perivascular lymphoid hyperplasia with secondary suppurative bronchiolitis and alveolar atelectasis.

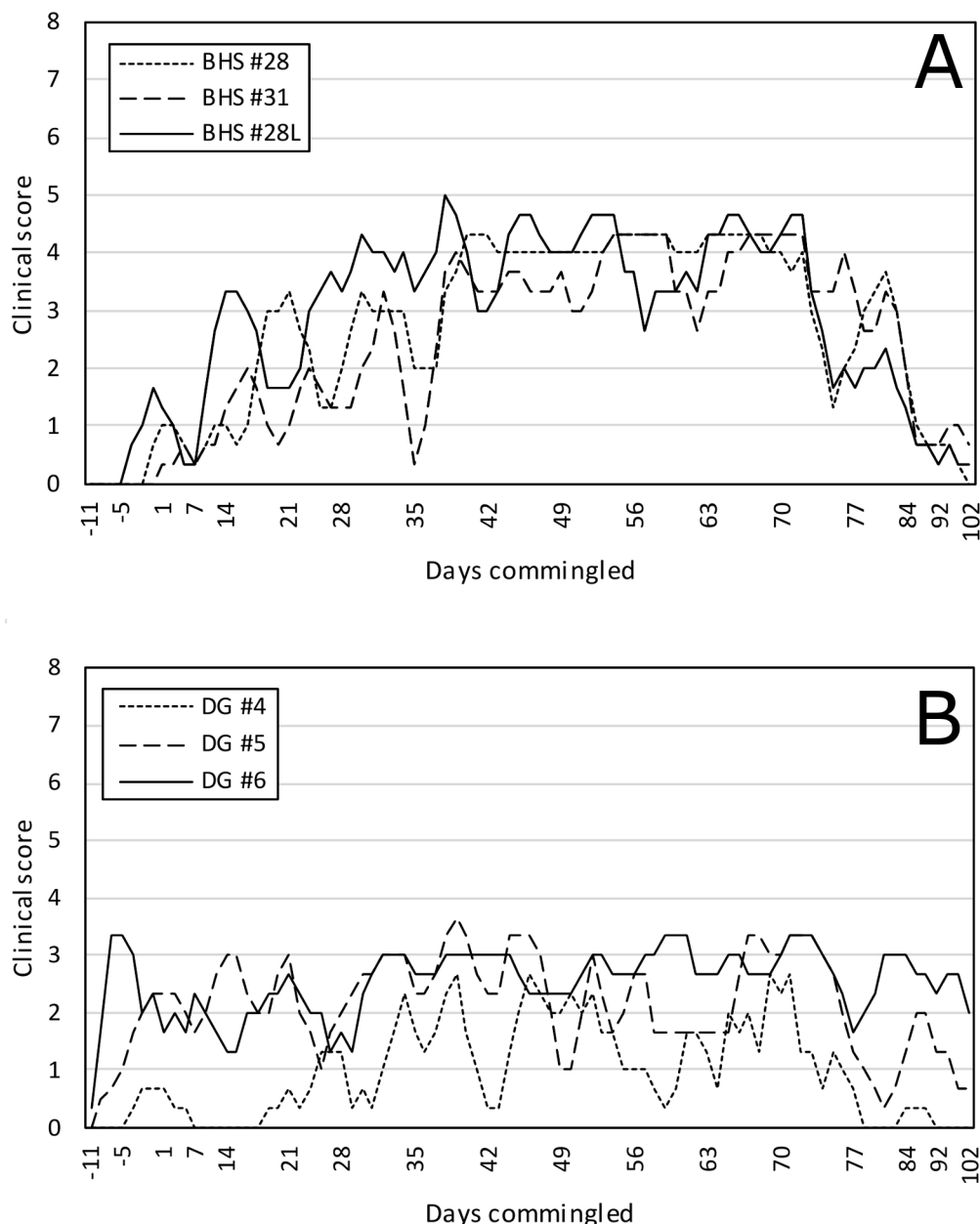


Fig 2. Clinical scores exhibited by commingled bighorn sheep and domestic goats in Experiment 3. Three-day rolling average summed clinical scores as described in Fig 1. (A) BHS; (B) DG. All BHS and DG in these experiments were naïve to *M. ovipneumoniae* prior to the experiment. DG4 and DG5 were exposed to *M. ovipneumoniae* on Day -11, and commingled with DG6 and BHS28, BHS28L and BHS31 on day 0.

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Microbiology findings

Microbiology and serology confirmed *M. ovipneumoniae* infection in all BHS only after contact with colonized DG, which resulted in seroconversion to this bacterium in both BHS and previously unexposed DG. *M. ovipneumoniae* IGS genotypes detected at necropsy in bighorn sheep or domestic goats shared identical sequences with those detected in the source goats prior to the studies. In experiment 1, a single IGS genotype was detected in all domestic goats prior to and after the experiment, and in all bighorn sheep at necropsy after the experiment. In

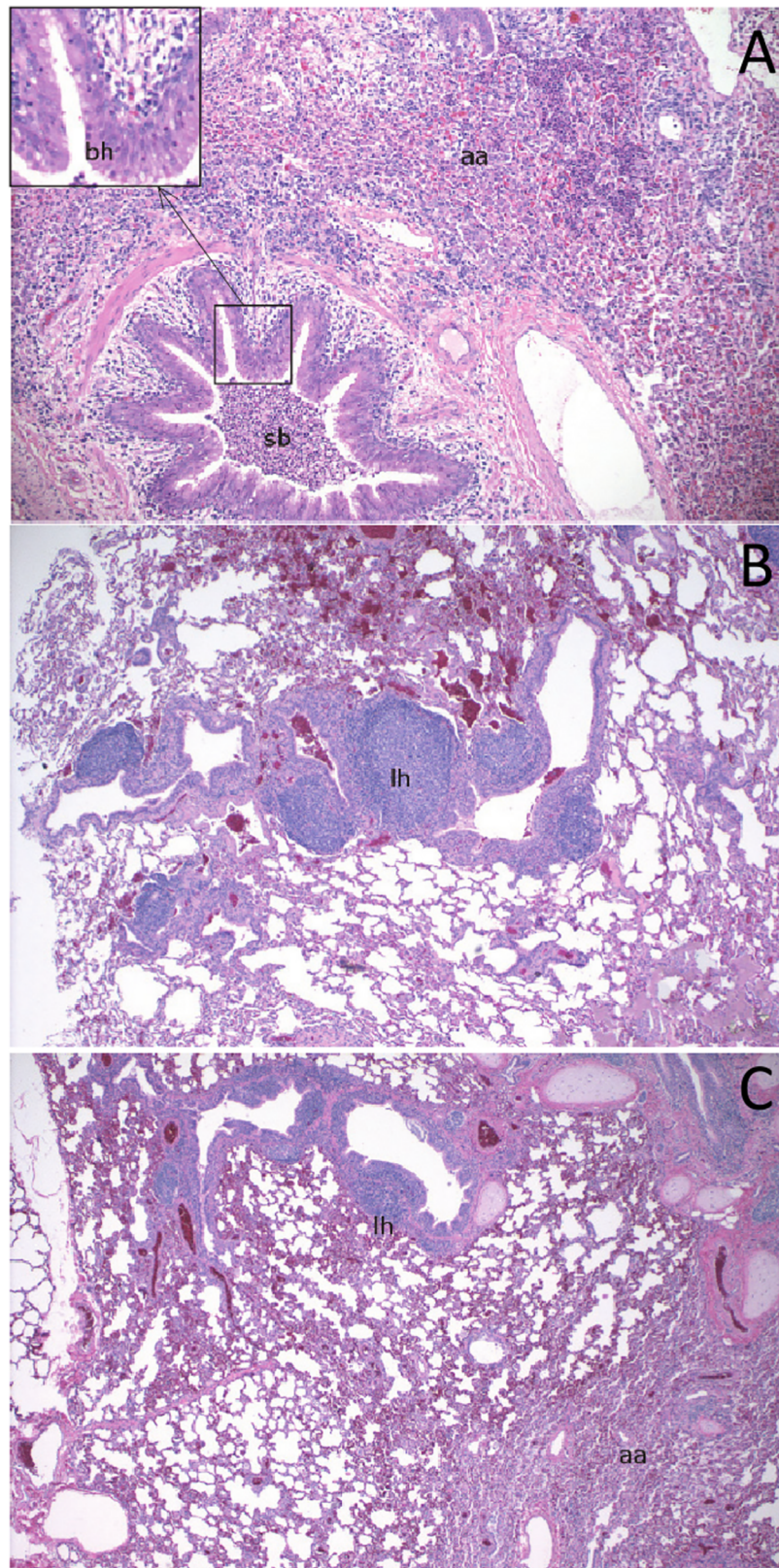


Fig 3. Representative histological lung lesions in experimental animals. Similar lesions were observed in all necropsied experimental animals. (A) BHS29 following experiment 1; (B) BHS28 following experiment 3,

and (C) DG5 following experiment 3. aa = alveolar atelectasis; bh = bronchiolar hypertrophy; lh = lymphoid hyperplasia; sb = suppurative bronchiolitis.

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experiment 2, two different IGS genotypes were detected in the domestic goat inoculum source, one of which was subsequently detected in all commingled goats and bighorn sheep, while the other strain was detected in all the domestic goats but was not detected in any of the comingled bighorn sheep. Both the bighorn sheep and domestic goat groups were colonized in the oropharynx by diverse Pasteurellaceae prior to, during, and after these experiments, and some of these bacteria were also isolated from pneumonic lung tissues of the animals at necropsy (Tables 1 and 3). These included *lktA*+ *M. haemolytica*, sometimes considered the most lethal Pasteurellaceae [23, 28].

Discussion

The results of this study both resemble yet substantially differ from those of previous studies of the impact of *M. ovipneumoniae* infection on initiation of pneumonia in bighorn sheep. Similar to previous studies exploring contacts with domestic sheep, comingling of *M. ovipneumoniae*-carrier domestic goats resulted in transmission of the pathogen to susceptible bighorn sheep, which then developed signs and lesions of respiratory disease [21]. However, contrary to previous studies that utilized domestic sheep carrying *M. ovipneumoniae*, the respiratory disease observed following experimental contact with domestic goats carrying *M. ovipneumoniae* was relatively mild, resulting in no fatalities. In addition, respiratory disease of comparable severity to that shown by the bighorn sheep also developed in naïve domestic goat hosts following infection, something that had not been reported in previous studies utilizing naïve domestic sheep. The lesions observed were consistent with those described in *Mycoplasma* spp. infections of sheep, goats and cattle, often called enzootic pneumonia. Enzootic pneumonia lesions are distinct from those caused by other bacterial agents of bronchopneumonia, such as *Pasteurella multocida* and *Mannheimia hemolytica*, which are characterized by widespread hemorrhage, necrosis and suppurative inflammation [13].

These contrasting results have interesting implications about the strain-specific virulence of *M. ovipneumoniae* and the specific roles of this and other bacterial pathogens in bighorn sheep respiratory disease. They also provide an area for additional research to identify management approaches for preventing new outbreaks of respiratory disease in bighorn sheep.

These results strengthen the previously documented links between exposure to *M. ovipneumoniae* and development of respiratory disease in bighorn sheep. All bighorn sheep exposed to goats carrying *M. ovipneumoniae* in experiments 1 and 3 developed signs and lesions of pneumonia, even when they had previously been exposed to the same goats in the absence of *M. ovipneumoniae*. All bighorn sheep kept in captivity under very similar conditions, but without contact with goats (experiment 1 pen 2) or in contact with *M. ovipneumoniae*-free goats (experiment 2, pen 1) did not develop signs of pneumonia. Pasteurellaceae bacteria carrying the gene encoding their principal virulence factor (*lktA* encoding leukotoxin) are another group of pathogens that have been hypothesized to play key roles in bighorn sheep pneumonia [23, 28], but both the bighorn sheep and the domestic goats used in these studies carried these pathogens prior to the experiments, and the presence of these pathogens were not clearly linked to the experimental outcomes or pathologic lesions.

Despite the consistent development of bighorn sheep pneumonia following contact with domestic goats carrying *M. ovipneumoniae*, the disease outcomes were more similar to those observed in the two previous experiments in which domestic goats were comingled with bighorn sheep and differed markedly from previous bighorn sheep-domestic sheep comingling

experiments [4, 5, 8]. Unfortunately, the *M. ovipneumoniae* carriage status of the goats used in those previous experiments was not tested and no clinical observation data were reported. The cumulative 98% mortality reported for bighorn sheep comingled with domestic sheep (88 deaths among 90 bighorn sheep in 11 experiments as reviewed in [4]) was significantly higher than either the mortality rates of those two previous goat commingling experiments (2-tailed Fisher's exact test, $P = 0.0014$ [5] and <0.0001 [8], respectively) or the two experiments reported here (each $P < 0.001$). Therefore, while bighorn sheep comingled with *M. ovipneumoniae* carrier goats consistently developed respiratory disease and pneumonia, mortality was a rare outcome compared to that observed when bighorn sheep were comingling with domestic sheep carrying *M. ovipneumoniae*.

One hypothesis that may explain the differing severity of outcome of bighorn sheep infected with *M. ovipneumoniae* originating from domestic goats is that *M. ovipneumoniae* with differing virulence traits may be harbored by these two host species. Emerging evidence supports the existence of distinct clades of *M. ovipneumoniae* from these two host species [7, 14]. A naturally occurring bighorn sheep pneumonia outbreak linked to *M. ovipneumoniae* belonging to the domestic goat clade, described in [7], resulted in approximately 30% pneumonia-associated mortality in the adult bighorn sheep, similar to that reported in [5] and towards the lower end of the range of mortality rates reported in pneumonia outbreaks affecting wild bighorn sheep [3, 29–32].

Since impaired recruitment due to *M. ovipneumoniae*-associated annual lamb pneumonia mortality is recognized as a key population-limiting effect of pneumonia in bighorn sheep [11], we anticipated that the lambs born to the ewes comingled with domestic goats in these experiments would provide insights into the effects of goat-origin *M. ovipneumoniae* on lambs. In both pens, lambs experienced a similar range of diseases but no bronchopneumonia was observed in any lamb, and the deaths in pen 1 occurred prior to the age at which lamb pneumonia typically is seen. Therefore, the experiments were not informative about lamb pneumonia resulting from *M. ovipneumoniae* carried by domestic goats, although the infections experienced by the pen 1 lambs clearly documented pathogen transmission among domestic goats, bighorn ewes, and bighorn lambs. Further studies will be needed to determine the persistent effects of goat-origin *M. ovipneumoniae* on bighorn sheep lambs in the years following initial exposure.

Conclusions and management implications: The severity of disease induced in bighorn sheep by the goat-origin *M. ovipneumoniae* strains in these experiments was relatively mild and transient. However, important questions remain: 1) Do other, more virulent strain-types exist within the goat *M. ovipneumoniae* clade? 2) Are goat-origin *M. ovipneumoniae* persistently carried by some bighorn sheep that recover from mild infections, as sheep-origin strains sometimes are? 3) If persistent carriers of goat-origin *M. ovipneumoniae* exist among bighorn ewes, are these strains subsequently transmitted to their lambs and do they induce lamb pneumonia? Answers to these questions will be relevant to the development of risk-appropriate management procedures to protect bighorn sheep from epidemic pneumonia.

Supporting information

S1 Table. Clinical scores of bighorn sheep and domestic goats.
(XLSX)

Author Contributions

Conceptualization: TEB EFC KAP WJF.

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Formal analysis: TEB.

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Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease

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ABSTRACT

Pneumonia of bighorn sheep (*Ovis canadensis*) is a dramatic disease of high morbidity and mortality first described more than 80 years ago. The etiology of the disease has been debated since its initial discovery, and at various times lungworms, *Mannheimia haemolytica* and other Pasteurellaceae, and *Mycoplasma ovipneumoniae* have been proposed as primary causal agents. A multi-factorial “respiratory disease complex” has also been proposed as confirmation of causation has eluded investigators. In this paper we review the evidence for each of the candidate primary agents with regard to causal criteria including strength of association, temporality, plausibility, experimental evidence, and analogy. While we find some degree of biological plausibility for all agents and strong experimental evidence for *M. haemolytica*, we demonstrate that of the alternatives considered, *M. ovipneumoniae* is the best supported by all criteria and is therefore the most parsimonious explanation for the disease. The strong but somewhat controversial experimental evidence implicating disease transmission from domestic sheep is consistent with this finding. Based on epidemiologic and microbiologic data, we propose that healthy bighorn sheep populations are naïve to *M. ovipneumoniae*, and that its introduction to susceptible bighorn sheep populations results in epizootic polymicrobial bacterial pneumonia often followed by chronic infection in recovered adults. If this hypothesized model is correct, efforts to control this disease by development or application of vectored vaccines to Pasteurellaceae are unlikely to provide significant benefits, whereas efforts to ensure segregation of healthy bighorn sheep populations from *M. ovipneumoniae*-infected reservoir hosts are crucial to prevention of new disease epizootics. It may also be possible to develop *M. ovipneumoniae* vaccines or other management strategies that could reduce the impact of this devastating disease in bighorn sheep.

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1. Introduction

Philosophers of science have long debated the process of causal inference, coming to a current consensus following the work of Karl Popper and others that proof of hypothesis is essentially unattainable, and that it is instead hypothesis falsification that drives scientific progress (Popper,

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1959). The cause of a disease has been defined as an event, condition, or characteristic that plays an essential role in producing the disease and, following Popper, the causal effect of any single factor can only be understood in relationship to conceivable alternatives (Rothman, 1986). Bighorn sheep pneumonia exemplifies the difficulty of causal inference even in the case of a dramatic infectious disease characterized by high morbidity and mortality, and the cause(s) of this disease have been subject to decades of debate and controversy. In this paper, we compare the evidence for several candidate causal microbial agents that have been proposed for epizootic pneumonia of bighorn sheep, including lungworms, Pasteurellaceae (especially *Mannheimia haemolytica*), and *Mycoplasma ovipneumoniae*, using widely accepted criteria for causality of disease, including strength of association, temporal relationships, biological plausibility, experimental evidence and analogy (Rothman, 1986).

1.1. Background

Bighorn sheep vanished from much of their historic range in North America during westward expansion in the early 20th century (Dice, 1919; Grinnell, 1928; Buechner, 1960). The precipitous decline in numbers, from 1.5–2 million in the 19th century to 15–18,000 in the United States by 1960 (Buechner, 1960) was not a unique phenomenon, as many other wildlife species' populations were similarly devastated during this era. However, the complete extirpation of bighorn sheep from much of their range, the slow rate of recovery despite intensive management efforts, and the recent listing of several U.S. populations as federally endangered (USFWS, 1998, 2000) sets them apart from most other North American ungulates.

As with other species of wildlife, market hunting and competition with livestock for forage contributed to the decline of bighorn sheep (Spencer, 1943; Buechner, 1960). However, an unusual correlation between the introduction of domestic sheep (*Ovis aries*) and the rapid disappearance of bighorn sheep was noted by early investigators (Grinnell, 1928; Schillenger, 1937; Marsh, 1938). Pneumonia was recognized as an important cause of the decline by the turn of the 20th century, and remains the most significant disease impeding recovery (Rush, 1927; Buechner, 1960; Gross et al., 2000; Cassirer and Sinclair, 2007). Pneumonia outbreaks in previously healthy bighorn sheep populations typically affect all ages of animals, result in 30–90% mortality, and are nearly always followed by at least several years of annual pneumonia outbreaks restricted to lambs that dramatically reduce population growth (Spraker et al., 1984; Ryder et al., 1992; Cassirer et al., 1996; George et al., 2008). Sporadic or continuous pneumonia events can persist in both adults and lambs in interconnected populations for many years, limiting population growth at best and potentially leading to extinction at worst (Cassirer and Sinclair, 2007).

Pathologic descriptions of bighorn sheep pneumonia were first provided in the first half of the 20th century (Rush, 1927; Marsh, 1938). Rush described a chronic pneumonia and noted lung adhesions, pus, “dark ulcers” and

“a white strip around the edge of the lobes” in sheep necropsied in an all-age pneumonia outbreak that killed about 75% of the population on a portion of the Sun River game range in Montana in 1925. Marsh described a chronic pneumonia associated with lungworms (*Protostrongylus stilesi*), *Corynebacterium* (now *Trueperella*) *pyogenes* and *Pasteurella* spp. in winter pneumonia mortalities on the Sun River game range and Glacier and Yellowstone National Parks in the 1920s and 1930s, and an acute bacterial pneumonia associated with *Pasteurella* spp. and *T. pyogenes* in a high mortality summer pneumonia event affecting 2- to 3-month-old lambs at the National Bison Range. A similar variety of pathologies and agents continues to be observed in bighorn sheep pneumonia cases. More recently, additional lesions including rhinitis, otitis media, sinusitis, tracheitis, pleuritis, broncholar hyperplasia, and bronchiectasis have been associated with this disease (Cassirer and Sinclair, 2007; Besser et al., 2008). *M. ovipneumoniae* and respiratory viruses, especially parainfluenza-3 and respiratory syncytial virus, have also been added to list of potential pathogens (Aune et al., 1998; Weiser et al., 2003; Rudolph et al., 2007; Besser et al., 2008).

While there has been a lack of clarity surrounding the pathogens associated with bighorn sheep pneumonia, experimental trials commingling bighorn and domestic sheep have had very clear results: nearly all bighorn sheep (88 of 90, 98%) commingled with domestic sheep in 11 published studies conducted between 1979 and 2009 died of pneumonia, while the domestic sheep remained healthy, as summarized in Besser et al. (2012a). In marked contrast, most (52 of 56, 93%) bighorn sheep survived similar commingling with non-ovine ungulates, including cattle, horses, deer, elk and llamas, also summarized in Besser et al. (2012a). Therefore, candidate agents enzootic in domestic sheep have been and remain a logical focus of etiologic investigations of bighorn sheep pneumonia.

The epidemiology of emerging diseases in wildlife is often unclear and causative agents are often elusive (McCallum and Dobson, 1995; Laurance et al., 1996). However, pneumonia in bighorn sheep is particularly unusual in that during nearly a century of investigation and despite the occurrence of disease outbreaks with dramatically high morbidity and mortality, no specific pathogen has been strongly or consistently associated with the disease. This rather confusing situation further led to development of the concept of a respiratory disease complex lacking a single causal agent and due instead to environmental or physiological stressors predisposing animals to invasion of the lungs with a combination of agents (Spraker et al., 1984; Rudolph et al., 2007). However, little evidence has been found for a correlative or causal relationship of physiological (Kraabel and Miller, 1997; Goldstein et al., 2005) or environmental stressors (Monello et al., 2001) and pneumonia in bighorn sheep. Thus a long history of conflicting and changing ideas about etiology and epidemiology and a lack of systematic examination of the evidence for causality have produced significant roadblocks to understanding and managing pneumonia in bighorn sheep.

2. The role of lungworms

Lungworms were one of the first agents proposed as causal agents for pneumonia in bighorn sheep. Two species of lungworm, *Protostrongylus ruscii* and *P. stilesi*, are native parasites of North American wild sheep. The worms have a multi-host life cycle that includes infection of various genera of land snails during larval stages. An early hypothesis about the etiology of bighorn sheep pneumonia was that lungworms (specifically *P. stilesi*) were primary agents that caused pneumonia directly or precipitated secondary bacterial infection in the lungs (Marsh, 1938). This disease was referred to as the bighorn sheep lungworm–pneumonia complex (Buechner, 1960; Forrester, 1971).

2.1. Strength of association

The association of lungworms with pneumonia is weak. The principal evidence in support of the lungworm hypothesis was the observation of lungworms and associated inflammation within pneumonic tissues of most affected bighorn sheep (Marsh, 1938; Forrester and Senger, 1964b; Forrester, 1971; Demartini and Davies, 1977). However, lungworm infestations are ubiquitous among wild sheep, including the northern thinhorn (Dall's) sheep (*Ovis dalli*) in which pneumonia epizootics have not been reported (Jenkins et al., 2007) as well as in most bighorn sheep populations unaffected by pneumonia (Forrester and Senger, 1964b; Hibler et al., 1982; Festa-Bianchet, 1991).

2.2. Plausibility

It is plausible that lungworms could predispose bighorn sheep to bacterial pneumonia. Lungworm larvae are frequently visible in pneumonic lung tissues of affected bighorn sheep, often adjacent to histopathologic lesions including bronchiolar hyperplasia and lymphocyte cuffing of airways commonly seen in bighorn sheep pneumonia. Furthermore, lungworm larvae are transmitted transplacentally to bighorn lambs, consistent with pre-weaning pneumonia mortality often seen in this disease (Forrester and Senger, 1964a; Hibler et al., 1974). However, bighorn sheep pneumonia lesions primarily affect anterior-ventral lung lobes while verminous pneumonia is expected to be broadly distributed with the most severe lesions usually occurring in the diaphragmatic lobes (Radostits et al., 2007). Finally, lungworms do not provide a plausible explanation for the well-documented transmission of pneumonia to bighorn sheep following contact with domestic sheep, given the different species of protostrongylids that typically affect these different host species (Panuska, 2006).

2.3. Experimental evidence

Experimental evidence is not supportive of the lungworm hypothesis. Experimental challenge of susceptible bighorn sheep with very high doses of infective lungworm larvae failed to produce any observed disease, refuting the possibility that bighorn sheep might be especially susceptible to lungworm induced pneumonia (Buechner,

1960; Forrester, 1971; Samson et al., 1987). Furthermore, anthelmintic treatments that effectively reduced lungworm burdens (Schmidt et al., 1979) were unsuccessful at improving lamb survival or preventing epizootics (Risenhoover et al., 1988; Goldstein et al., 2005).

Perhaps reflecting this evidence, by the 1980s the model of the lungworm–pneumonia complex had changed to describe a disease in which lungworm infection primarily affected lambs as a consequence of poor habitat conditions and other stressors (Spraker et al., 1984; Risenhoover et al., 1988; Easterly et al., 1992).

3. The role of Pasteurellaceae

M. haemolytica, *Bibersteinia trehalosi* (formerly designated *Pasteurella haemolytica* types A and T, respectively) and *P. multocida*, all commonly associated with pneumonia of domestic ruminants (Griffin et al., 2010), have been frequently isolated from the lungs of bighorn sheep with pneumonia and have been implicated as causal agents since the 1920s (Rush, 1927; Queen et al., 1994; Weiser et al., 2003; Rudolph et al., 2007). Among these bacteria, most research attention has been directed to leukotoxin positive strains of *M. haemolytica*, a bacterium hypothesized to be transmitted from domestic sheep to bighorn sheep and involved in the etiology of the disease, although the extent of the hypothesized involvement has ranged from “contribute to a pneumonia complex. . .” (Wild and Miller, 1991) to “consistently causes fatal bronchopneumonia in bighorn sheep” (Herndon et al., 2011). Other aerobic bacteria are also frequently isolated from pneumonic bighorn sheep lungs, including *T. pyogenes* (particularly from bighorn sheep with chronic lesions and/or pulmonary abscessation) (Queen et al., 1994) and an as yet unnamed *Moraxella* sp. (unpublished data, GenBank JQ814872), but these have generally been considered incidental findings rather than causative agents.

3.1. Strength of association

Strength of association is weak. Prevalence of specific Pasteurellaceae pathogens in lung tissues of pneumonic bighorn sheep, as detected by bacterial cultures or by polymerase chain reaction (PCR) tests, though generally high, is similar to that detected in lungs of healthy bighorn sheep that have died from causes other than pneumonia, and therefore do not reflect strong association with disease (Cassirer, 2005; Kelley et al., 2007; Besser et al., 2012b). Leukotoxin positive *M. haemolytica* can be detected, albeit at lower frequency than in domestic sheep, in the nasopharyngeal flora of healthy bighorn sheep in populations unaffected by pneumonia (Foreyt and Lagerquist, 1996; Kelley et al., 2007; Tomassini et al., 2009). Using culture and PCR, Besser et al. (2012b) documented frequent detection of *M. haemolytica* (57%), *B. trehalosi* (77%) and *P. multocida* (48%) in the lung tissues of 44 pneumonic animals from eight bighorn sheep pneumonia outbreaks in Montana, Nevada, Oregon, and Washington. Normal lung tissues from five bighorn sheep in healthy populations showed similar prevalence of the same three Pasteurellaceae (60%, 80% and 20%, respectively) using the same methodologies.

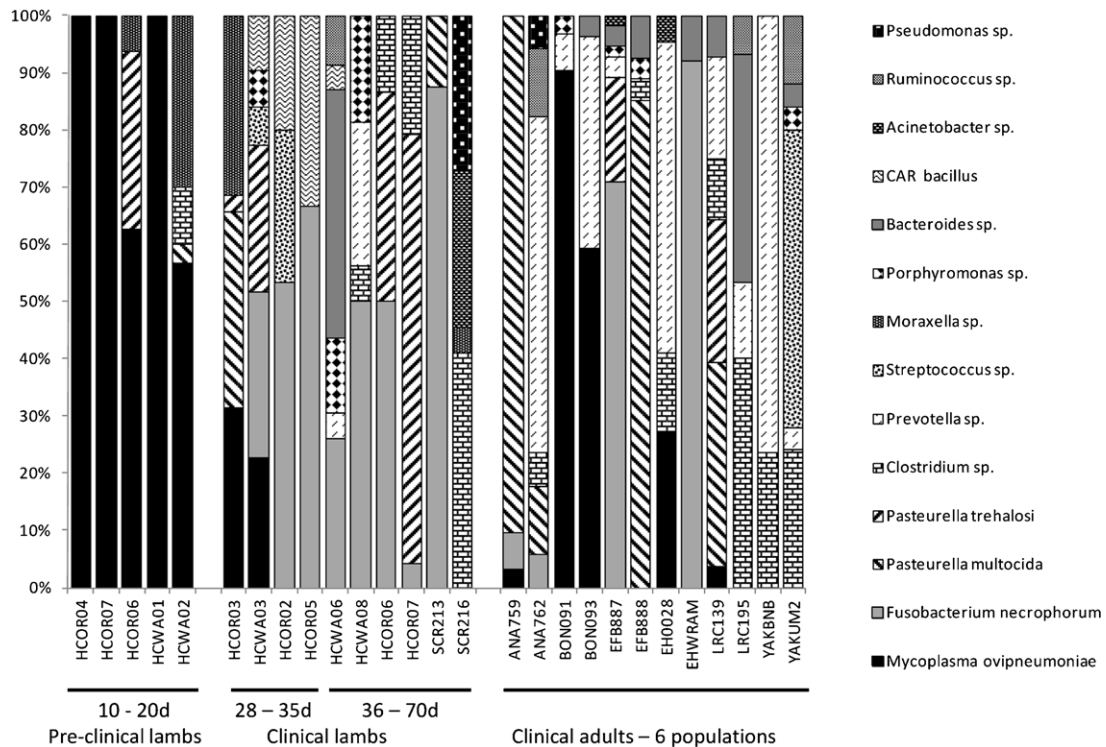


Fig. 1. Principal bacterial components of pneumonic bighorn sheep lung tissues based on 16S rDNA clone library analysis (Besser et al., 2008, 2012b). The X-axis legend specifies populations of origin of the bighorn sheep from which the tissues were sampled: HCOR = Hell's Canyon OR; HCWA = Hell's Canyon, WA; SCR = Spring Creek SD; ANA = Anaconda MT; BON = Bonner MT; EFB = East Fork Bitterroot MT; EH = East Humboldt NV; LRC = Lower Rock Creek MT; YAK = Yakima WA.

Culture independent assessment of the bacterial flora present in affected lung tissues of two bighorn sheep from each of the eight outbreak populations quantified the percentage of Pasteurellaceae-specific ribosomal 16S operons within the lung lesions: *P. multocida*, 12.7%; *B. trehalosi*, 8.7%; and *M. haemolytica*, 0.2% (Besser et al., 2008, 2012b) (Fig. 1). These data indicate that while these agents are frequently detectable in affected animals, they are relatively (*P. multocida*, *B. trehalosi*) or extremely (*M. haemolytica*) minor constituents of the infections. While this does not exclude the possibility that these agents may have been present in higher numbers in earlier stages of the disease, it is notable that their frequency did not differ between animals collected by gunshot early in the disease course and animals that died of the disease (Besser et al., 2012b).

3.2. Plausibility

As well established respiratory pathogens in many other animal hosts, Pasteurellaceae are generally plausible as causal agents of pneumonia in bighorn sheep. They also exhibit characteristics consistent with the long established association of bighorn sheep pneumonia with domestic sheep contact: bighorn sheep predominantly carry leukotoxin negative *B. trehalosi*, whereas most domestic sheep carry leukotoxin-expressing *M. haemolytica* (Sweeney et al., 1994; Jaworski et al., 1998). Therefore, the observed association of bighorn sheep pneumonia following domestic sheep contact is plausibly consistent with transmission

of leukotoxin expressing Pasteurellaceae, particularly *M. haemolytica*. On the other hand, given the highly epizootic nature of this disease, a causal agent isolated from multiple affected animals within an outbreak would be expected to be indistinguishable by appropriate molecular epidemiologic 'fingerprinting' genetic tests. In a few cases, indistinguishable strain types (or strains carrying unique artificial markers) have been demonstrated in bighorn sheep experimentally commingled with domestic sheep or goats, consistent with transmission (Rudolph et al., 2003; Kelley et al., 2007; Lawrence et al., 2010). However, in general, indistinguishable (epizootic) Pasteurellaceae strain types have not been identified either within natural outbreaks (Weiser et al., 2003; Besser et al., 2012b) or in outbreaks following experimental commingling of domestic sheep and bighorn sheep (Onderka and Wishart, 1988; Foreyt, 1990, 1994; Foreyt, 1998), calling into question Pasteurellaceae as epizootic disease agents.

3.3. Experimental evidence

The strongest evidence for the hypothesis that bighorn sheep pneumonia is caused by Pasteurellaceae is the rapidly lethal bronchopneumonia that frequently results from experimental challenge of bighorn sheep with leukotoxin positive *M. haemolytica* or *B. trehalosi* (Onderka et al., 1988; Foreyt et al., 1994; Kraebel et al., 1998). However, other experimental evidence does not unequivocally support this hypothesis. For example, commingling of bighorn

sheep and domestic cattle did not result in epidemic pneumonia (Foreyt, 1994; Foreyt and Lagerquist, 1996), despite the high prevalence of carriage by cattle of *lktA* positive *M. haemolytica* types A1 and A2, both highly lethal to bighorn sheep (Dassanayake et al., 2009; Foreyt and Lagerquist, 1996). Similarly, commingling bighorn sheep with deer or elk that were pharyngeal carriers of '*P. haemolytica*' or *P. multocida* did not result in any clinical disease over a six-month period (Foreyt, 1992). Furthermore, although sporadic pneumonia deaths attributed to *M. haemolytica* were observed in bighorn sheep experimentally commingled with non-ovine domestic ruminants carrying *M. haemolytica*, epizootic disease did not occur (Foreyt, 1994; Foreyt et al., 2009; Besser et al., 2012a).

It should be noted that *Pasteurella multocida*, a major respiratory pathogen of sheep, cattle, swine, and many non-ungulate species (Harper et al., 2011) and frequently isolated from bighorn sheep with pneumonia (Aune et al., 1998; Weiser et al., 2003; Rudolph et al., 2007), has as yet received relatively little research attention for its potential role in bighorn sheep pneumonia. *P. multocida* was frequently detected in pneumonic bighorn sheep involved in five of the eight outbreaks investigated by Besser et al. (2012b), was relatively uncommonly detected in lung tissues of healthy control animals (20%), and was the sole Pasteurellaceae for which some clonal strain types were observed within multiple animals within outbreaks (Besser et al., 2012b). To our knowledge, the effects of experimental challenge of bighorn sheep with *P. multocida* have not been reported. Therefore, more evidence is needed to critically assess the possible causal role of this bacterium in bighorn sheep pneumonia.

4. The role of *M. ovipneumoniae*

Initial observations of *M. ovipneumoniae* in bighorn sheep were made in pneumonic animals from the Black Mountains in Arizona in 1980 (Bunch et al., 1985). This agent was then reported in association with a pneumonia epizootic affecting Dall's sheep in a zoo (Black et al., 1988). Subsequently, *M. ovipneumoniae* was detected in animals removed from Hells Canyon during an epizootic in the mid-1990s (Rudolph et al., 2007). However, prior to development and widespread use of molecular detection techniques, *M. ovipneumoniae* received little attention, probably because its fastidious nature rendered it difficult to isolate in culture (Weiser et al., 2012).

4.1. Strength of association

M. ovipneumoniae is strongly associated with pneumonia in bighorn sheep. The strength of association of *M. ovipneumoniae* was first investigated in a study of bighorn lambs collected in Hells Canyon (Besser et al., 2008). In addition to documenting the presence of *M. ovipneumoniae* in all pneumonic lambs but not in a healthy lamb, the study also included a serosurvey of nine additional pneumonic bighorn sheep populations, all of which exhibited high seroprevalence, and nine healthy bighorn sheep populations, all of which were seronegative. Subsequently, *M. ovipneumoniae* was detected in pneumonic lung tissues of

>95% of 44 affected bighorn sheep lungs sampled in eight pneumonia epizootics that occurred in the western US during 2009–2010 but was absent in lung tissues of animals ($N=5$) obtained from two populations unaffected by pneumonia (Besser et al., 2012b). Table 1 lists evidence of *M. ovipneumoniae* presence as tested by culture, PCR, or specific serologic antibodies in bighorn sheep populations of known health status from across western North America. Exposure to *M. ovipneumoniae* was documented in all 36 populations tested that were classified as pneumonic by the wildlife officials submitting the diagnostic specimens, but in only 3 of 32 populations that were classified as healthy ($P<0.001$; odds ratio ∞ /undefined; 95% confidence interval = 31 – ∞).

4.2. Temporality

As reported above, in recent years since reliable diagnostic tools have been available, we have not identified any bighorn sheep pneumonia outbreak in which *M. ovipneumoniae* was not detected in a high proportion of affected animals. In the retrospective serologic study reported in Besser et al. (2008), three populations were identified that remained seronegative up to the year preceding the first recognized pneumonia outbreak, following which all three populations developed high seroprevalence of *M. ovipneumoniae* specific antibody. Similarly, animals in the Asotin Creek population of Hells Canyon were repeatedly tested and remained seronegative and culture/PCR negative for *M. ovipneumoniae* prior to its first pneumonia outbreak in 2012, during which animals with pneumonia were PCR positive for *M. ovipneumoniae* and subsequent to which, surviving animals demonstrated seroconversion.

4.3. Plausibility

The biologic plausibility of a role for *M. ovipneumoniae* is addressed by several lines of evidence, including the role of a natural host (domestic sheep) as a risk factor for disease transmission, the nature of the lesions observed in affected bighorn sheep, and the molecular epidemiologic evidence of epizootic transmission of strains of this agent in disease outbreaks. The host range of *M. ovipneumoniae* is limited to Caprinae and the species colonizes domestic sheep flocks at high prevalence (Nicholas et al., 2008). As a result, domestic sheep, domestic goats, and mouflon are plausible biologic sources of this agent, whereas other ungulates, including domestic cattle, horses, llamas and wild Cervidae, are not. Purulent otitis media, a non respiratory lesion often observed in bighorn sheep with pneumonia (Besser et al., 2008), is also frequently associated with respiratory *Mycoplasma* infections in other hosts. Chronic bronchointerstitial pneumonia with lymphocytic cuffing of airways and bronchiolar hyperplasia are often observed in the lungs of bighorn sheep, typical of lesions observed in other respiratory *Mycoplasma* infections (Ettorre et al., 2007; Opriessnig et al., 2011). The lung histopathology of pneumonic bighorn sheep may also include acute fibrinous bronchopneumonia and pleuritis typical of Pasteurellaceae and different individuals from within the same outbreak may present with either

Table 1*M. ovipneumoniae* (Movi) status of bighorn sheep populations based on serology and/or culture/PCR.

Pneumonic populations			Non-pneumonic populations		
Population	State	Movi status	Population	State	Movi status
Kanab Creek	AZ	Positive	Cadomin	AB	Negative
E. Fraser Valley	BC	Positive	Ram Mt	AB	Negative
Peninsular	CA	Positive	Kofa	AZ	Positive
White Mts	CA	Positive	Navaho	AZ	Negative
Fossil Ridge	CO	Positive	Spence's Bridge	BC	Negative
Redbird	ID	Positive	Sierra Nevada	CA	Negative
L. Salmon River	ID	Positive	Wild Horse Island	MT	Negative
Morgan Cr	ID	Positive	Missouri Breaks	MT	Positive
Anaconda	MT	Positive	Sun River (to 2011)	MT	Positive
Bonner	MT	Positive	San Francisco River	NM	Negative
Cinnabar Basin	MT	Positive	Bare Mtns	NV	Negative
E.F. Bitterroot	MT	Positive	Montana Mtns	NV	Negative
Lower Rock Cr	MT	Positive	Monte Cristo Mtns	NV	Negative
Upper Rock Cr	MT	Positive	Muddy Mtns	NV	Negative
Sun River (since 2011)	MT	Positive	Pine Forest	NV	Negative
San Andreas	NM	Positive	River Mtns	NV	Negative
E. Humboldt	NV	Positive	Stonewall Mtns	NV	Negative
Hays Canyon	NV	Positive	Coglan Butte	OR	Negative
Pancake Range	NV	Positive	Imnaha (to 1995)	OR	Negative
Ruby Mtns	NV	Positive	Lostine (to 1986)	OR	Negative
Snowstorm Mtns	NV	Positive	Lookout	OR	Negative
Pilot/Leppy Hills	NV/UT	Positive	Badlands (south)	SD	Negative
Imnaha (since 1996)	OR	Positive	Clemans Mt	WA	Negative
Lostine (since 1987)	OR	Positive	Asotin Cr (to 2012)	WA	Negative
Sheep Mt	OR	Positive	Manson	WA	Negative
Muir	OR	Positive	Mt Hull	WA	Negative
Custer State Park	SD	Positive	Palmer Lake	WA	Negative
Hill City	SD	Positive	Quilomene	WA	Negative
Rapid Cr	SD	Positive	Sinlahekin	WA	Negative
Spring Cr	SD	Positive	Swakane	WA	Negative
Asotin Cr (since 2012)	WA	Positive	Tieton	WA	Negative
Black Butte	WA	Positive	Tucannon	WA	Negative
Wenaha	WA	Positive			
Yakima	WA	Positive			
Gros Ventre	WY	Positive			
Whiskey Basin	WY	Positive			

acute or chronic lesions or in some cases, the chronic lesion may be seen in different areas of the same lungs that exhibit the acute lesion (Besser et al., 2008; unpublished data). The inconsistent presence of acute lung lesions plausibly reflects the variable presence of secondary bacterial pathogens known to induce acute lesions (such as Pasteurellaceae), while the chronic lesions may reflect underlying *M. ovipneumoniae* disease. The pathophysiology of *M. ovipneumoniae* infection is believed to result from tracheal and bronchial ciliostasis and ciliary degeneration that diminish the ability of the infected animal to clear inhaled bacteria (Niang et al., 1998; Alley et al., 1999). The result of impaired mucociliary clearance is polymicrobial pneumonia, consistent with the diverse bacterial populations infecting lung tissues detected by the 16S studies described earlier (Fig. 1 and Fig. S1; Besser et al., 2008, 2012b). Finally, epizootic transmission of *M. ovipneumoniae* within bighorn sheep outbreaks has been confirmed by molecular epidemiology: indistinguishable 16S–23S intergenic spacer region DNA sequences were observed in animals within each of eight outbreaks, while strain types generally differed between outbreaks (Besser et al., 2012b).

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.prevetmed.2012.11.018>.

4.4. Experimental evidence

The role of *M. ovipneumoniae* in bighorn sheep pneumonia has also been tested experimentally both by exclusion and by introduction. As described earlier, previously published studies of bighorn sheep commingled with domestic sheep or mouflon reported very high (98%) pneumonia mortality within 90 days. In marked contrast and supportive of a key causal role for *M. ovipneumoniae*, three of four bighorn sheep survived commingling with *M. ovipneumoniae*-free domestic sheep for 100 days (Besser et al., 2012a). Experimental challenges of bighorn sheep with *M. ovipneumoniae* have produced more varied results. An isolate given to 1-week-old bighorn lambs failed to produce significant clinical disease: one of two challenged lambs developed a strong anti-*M. ovipneumoniae* antibody response and had focal chronic pneumonia but was culture negative at necropsy, while the other challenged lamb remained seronegative but culture positive from both the upper and lower respiratory tract through the >60 day experiment (Besser et al., 2008). Due to the possibility of in vitro attenuation of the Mycoplasma during the isolation process (Buddle et al., 1984), subsequent *M. ovipneumoniae* challenges by our laboratory have utilized nasopharyngeal washes collected directly from

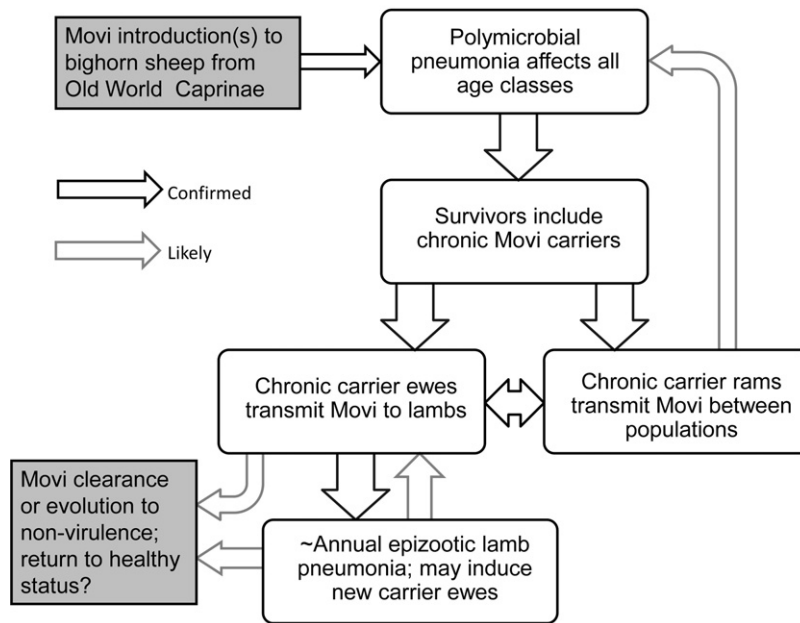


Fig. 2. Model diagram of the *M. ovipneumoniae* (Movi) hypothesis as presented in this paper.

M. ovipneumoniae colonized animals then treated by incubation (3 h, 35°C) with ceftazidime to eliminate Pasteurellaceae and other susceptible bacteria. These challenges have consistently produced signs of respiratory tract disease and pneumonia, including coughing, nasal discharge, ear droop, and head shaking similar to those seen in the natural disease. In one published study, four bighorns challenged in this manner developed clinical signs consistent with pneumonia, and one animal died during the 70 days that the animals were followed after challenge (Dassanayake et al., 2010). In subsequent experiments, transmission of *M. ovipneumoniae* infection from single infected animals to all in-contact animals and to all animals in adjacent pens was demonstrated in two separate experiments involving a total of nine bighorn sheep. All bighorn sheep infected by *M. ovipneumoniae* developed bronchopneumonia and several died (unpublished observations).

4.5. Analogy

Infection of swine with the closely related bacterium (*M. hyopneumoniae*) is similarly associated with 'atypical pneumonia' that results in a similar chronic lesion and that is similarly characterized by polymicrobial infection with a broad spectrum of inhaled oral and pharyngeal bacteria (Opriessnig et al., 2011).

5. Hypothesis: a conceptual model of etiology of bighorn sheep pneumonia

As reviewed earlier, among the many pathogens associated with bighorn sheep pneumonia, *M. ovipneumoniae* most completely satisfies accepted criteria for a causal role in disease, including strength of association, temporal

relationship, biological plausibility, experimental evidence and analogy (Rothman, 1986). These data suggest a new model (Fig. 2), in which a bacterium (*M. ovipneumoniae*) native to domestic sheep and goats but previously exotic to North American wild sheep, plays a key role: Epizootic all-ages pneumonia ensues when this agent is transmitted into a naive bighorn sheep population, as the bacterium interferes with normal bronchociliary clearance mechanisms (Niang et al., 1998) leading to polymicrobial pneumonia. The polymicrobial pneumonia frequently includes obligate anaerobic bacteria, Pasteurellaceae, *T. pyogenes*, and diverse other taxa. The case fatality rate of the pneumonia may be affected by the dose or the virulence of the infecting *M. ovipneumoniae* strain, the nature of the secondary bacterial invaders, the resistance of the host, or other as yet unidentified factors. Animals surviving the initial epizootic can become chronic carriers, shedding *M. ovipneumoniae* in the nasal secretions. Chronic carrier ewes may transmit the infection to subsequent years' lambs resulting in epizootic lamb pneumonia while chronic carrier rams may potentially spread the agent to adjacent populations in the course of their natural movements (Geist, 1971; Singer et al., 2000).

Once introduced into a bighorn sheep population, *M. ovipneumoniae* may be cleared after a year or two, or persist for decades. Potential ultimate outcomes of infection include selection for attenuation of the agent due to the high death loss of hosts infected with fully virulent strains, selection of bighorn sheep with increased genetic resistance to the infection, stochastic loss of carrier animals resulting in elimination of the agent and cessation of the infectious cycle, or local extirpation of bighorn sheep populations due to lack of lamb survival. Research is needed to better describe the range of

outcomes observed following bighorn sheep pneumonia and to determine which of the above mechanisms is at play.

If this hypothesized model is correct, efforts to control this disease by development or application of vectored vaccines to Pasteurellaceae are unlikely to provide significant beneficial effects, whereas efforts to ensure segregation of healthy bighorn sheep populations from *M. ovipneumoniae*-carrying domestic sheep, domestic goats, and chronically infected bighorn sheep are crucial to prevent new disease epizootics. In addition, it may be possible to develop *M. ovipneumoniae* vaccines or other management procedures that could reduce shedding of the agent by domestic small ruminants in order to reduce the risk of transmission to bighorn sheep. An effective vaccine could also potentially be usefully applied to bighorn sheep to reduce disease morbidity and mortality if the challenges inherent in vaccinating wildlife, particularly a species that inhabits inaccessible terrain, could be overcome.

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Special Section on Mountain Sheep Management

Pneumonia in Bighorn Sheep: Risk and Resilience

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ABSTRACT Infectious disease contributed to historical declines and extirpations of bighorn sheep (*Ovis canadensis*) in North America and continues to impede population restoration and management. Reports of pneumonia outbreaks in free-ranging bighorn sheep following contact with domestic sheep have been validated by the results of 13 captive commingling experiments. However, ecological and etiological complexities still hinder our understanding and control of respiratory disease in wild sheep. In this paper, we review the literature and summarize recent data to present an overview of the biology and management of pneumonia in bighorn sheep. Many factors contribute to this population-limiting disease, but a bacterium (*Mycoplasma ovipneumoniae*) host-specific to Caprinae and commonly carried by healthy domestic sheep and goats, appears to be a primary agent necessary for initiating epizootics. All-age epizootics are usually associated with significant population declines, but mortality rates vary widely and factors influencing disease severity are not well understood. Once introduced, *M. ovipneumoniae* can persist in bighorn sheep populations for decades. Carrier females may transmit the pathogen to their susceptible lambs, triggering fatal pneumonia outbreaks in nursery groups, which limit recruitment and slow or prevent population recovery. The demographic costs of disease persistence can be equal to or greater than the impacts of the initial epizootic. Strain typing suggests that spillover of *M. ovipneumoniae* into bighorn sheep populations from domestic small ruminants is ongoing and that consequences of spillover are amplified by movements of infected bighorn sheep across populations. Therefore, current disease management strategies focus on reducing risk of spillover from reservoir populations of domestic sheep and goats and on limiting transmission among bighorn sheep. A variety of techniques are employed to prevent contacts that could lead to transmission, including limiting the numbers and distribution of both wild and domestic species. No vaccine or antibiotic treatment has controlled infection in domestic or wild sheep and to date, management actions have been unsuccessful at reducing morbidity, mortality, or disease spread once a bighorn sheep population has been exposed. More effective strategies are needed to prevent pathogen introduction, induce disease fadeout in persistently infected populations, and promote population resilience across the diverse landscapes bighorn sheep inhabit. A comprehensive examination of disease dynamics across populations could help elucidate how disease sometimes fades out naturally and whether population resilience can be increased in the face of infection. Cross-jurisdictional adaptive management experiments and transdisciplinary collaboration, including partnerships with members of the domestic sheep and goat community, are needed to speed progress toward sustainable solutions to protect and restore bighorn sheep populations. © 2017 The Wildlife Society.

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Infectious disease has influenced bighorn sheep (*Ovis canadensis*) population dynamics at least since the westward expansion of the United States, and plausibly since the Spanish colonization of Mexico and the American Southwest. The importance of disease in the historical decline and extirpation of bighorn sheep across much of their range from southern Canada to Mexico is unique among North American ungulates. Early naturalists described catastrophic die-offs and suggested that disease outbreaks and disappearance of wild sheep might be attributed to the introduction of domestic sheep and goats into bighorn sheep range (Brooks 1923, Grinnell 1928). Shillinger (1937) reported on an experiment in which Rocky Mountain bighorn sheep (*O. c. canadensis*), after surviving well in captivity by themselves, all died when healthy-appearing domestic sheep were introduced to the enclosure. Shillinger speculated “The only evident explanation is that some infectious organism well tolerated by the domestic sheep. . . was transferred to the wild animals with disastrous results” (Shillinger 1937:301). Since then, many more disastrous disease outbreaks have occurred in free-ranging wild sheep populations and another 12 domestic-wild sheep commingling experiments have been conducted with similar deadly results for bighorn sheep (Wehausen et al. 2011, Besser et al. 2012a). Together these observations have culminated in the recognition that management of bighorn sheep also involves management of pathogen transmission from domestic sheep (Council for Agricultural Science and Technology 2008, Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012, The Wildlife Society 2015).

The susceptibility of bighorn sheep to infectious agents carried by domestic sheep is not unexpected given that genetic similarity with domestic hosts is a key risk factor for pathogen spillover and associated disease-induced population declines in wildlife (Pedersen et al. 2007). The bighorn sheep is the only North American ungulate with a congeneric domesticated relative. Although species divergence occurred over a million years ago (Rezaei et al. 2010), domestic and bighorn sheep are still sufficiently similar that they can interbreed and produce viable offspring (Young and Manville 1960). Bighorn and domestic sheep and goats share lineages of immune-associated genes in the major histocompatibility complex (Gutierrez-Espeleta et al. 2001), but inherent differences in immune systems likely contribute to the disparity in effects of pathogens across species (Silflow et al. 1989, Dassanayake et al. 2009, Highland et al. 2016).

Understanding and acknowledging the importance of pathogen spillover from domestic sheep and goats has provided valuable perspective and direction for management of respiratory disease in bighorn sheep and, at the same time, has complicated it. Wildlife biologists managing bighorn sheep are now faced with an uncomfortable choice between promoting connectivity and gene flow to restore remnant populations and increasing fragmentation and limiting dispersal to reduce the risk of pathogen spillover and transmission. The impact of disease persistence in the aftermath of all-age epizootics is also a serious obstacle to population management. In this paper, we review the

literature and include a synthesis of data from our respective jurisdictions and from members of the Western Association of Fish and Wildlife Agencies Wild Sheep Working Group to provide an overview of the current state of knowledge about pneumonia in bighorn sheep. We report on impacts to individuals and populations, describe current management directions, and discuss potential strategies for moving forward.

CAUSES OF PNEUMONIA IN BIGHORN SHEEP

Pneumonia in bighorn sheep is a microbiologically complex disease, and many diverse bacteria are detected in the lungs of fatally affected animals, including pathogens that cause pneumonia and other diseases in livestock such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Fusobacterium necrophorum*. Some of these pathogens are toxigenic and lethal to captive bighorn sheep in experimental trials (Foreyt et al. 1994, Dassanayake et al. 2009), but they do not exhibit a clear and consistent association with disease epizootics in free-ranging populations (Singer et al. 2000b, Weiser et al. 2003, Rudolph et al. 2007, Besser et al. 2012b, Shanthalingam et al. 2016). Over time, paradigms of disease etiology have shifted, reflecting the diversity of pathogens and nonpathogenic agents detected in the lungs of pneumonic bighorn sheep. Suspected causes have ranged from lungworm infection (*Protostrongylus* spp.) to leukotoxin positive Pasteurellaceae, to a multi-factorial respiratory disease complex (Besser et al. 2013). Much attention has focused on virulent Pasteurellaceae bacteria where problems with accurate detection and classification have also complicated efforts to establish an association with pneumonia outbreaks in wild sheep (Angen et al. 2002, Walsh et al. 2012, Miller et al. 2013, Shanthalingam et al. 2014, Walsh et al. 2016).

In 2006, by applying culture-independent methods to high-quality samples of the lung microbiome obtained from free-ranging bighorn lambs in early stages of disease, researchers discovered that *Mycoplasma ovipneumoniae* was the pathogen that first invaded the lungs and predisposed affected animals to polymicrobial pneumonia (Besser et al. 2008). This pathogen does not act alone but appears to be a necessary agent for initiating epizootics. Further research is needed on the role of co-infection by known and perhaps as yet unrecognized pathogens as well as other factors that may contribute to disease outcomes by affecting transmission, carriage, and immunity (Dassanayake et al. 2010, Besser et al. 2012b, Fox et al. 2015, Wolff et al. 2016). Clarity on the significance of these interactions will help provide a more complete understanding of the variation observed in the course of infection and disease. We focus our discussion of microbial etiology on *M. ovipneumoniae* because, based on the experimental and empirical data which we review here, it currently presents the most parsimonious and well-supported model for a primary agent of bighorn sheep respiratory disease. For this reason it is also an important focus for management.

M. ovipneumoniae better meets Hill's (1965) causal criteria relevant to infectious diseases: strength of association,

temporality, plausibility, experimental evidence, and analogy than any competing proposed etiology (Besser et al. 2013). *M. ovipneumoniae* also fulfills Koch's postulates (Evans 1976, Walker et al. 2006) for a primary causal agent, with minor modifications. The strong association with disease (i.e., Koch's first postulate) is one of the most convincing lines of evidence for *M. ovipneumoniae*. Besser et al. (2013) detected *M. ovipneumoniae* in all free-ranging bighorn sheep populations affected by pneumonia epizootics where samples were available for testing ($n = 36$) and 91% (29/32) of bighorn sheep populations unaffected by pneumonia lacked evidence of exposure. Pneumonia outbreaks were associated with introduction of *M. ovipneumoniae* in ≥ 10 previously unexposed free-ranging bighorn sheep populations where testing was done before and after the epizootic (Besser et al. 2008, Bernatowicz et al. 2016; M. Cox, Nevada Department of Wildlife, unpublished data; J. Kanta, South Dakota Game, Fish, and Parks, unpublished data; J. Shannon, Utah Division of Wildlife, unpublished data; L. Jones, U.S. Fish and Wildlife Service, unpublished data). Limited information also suggests that free-ranging Dall's sheep (*Ovis dalli*) in Alaska and bighorn sheep populations in northern Alberta where bacterial pneumonia epizootics are not reported, have not been exposed to *M. ovipneumoniae* (Zarnke and Soren 1989, Besser et al. 2013).

Equally compelling, in 2 recent experiments 5 of 6 bighorn sheep survived when commingled with domestic sheep in the absence of *M. ovipneumoniae* (Besser et al. 2012a, Kugadas 2014). In contrast, virtually no (2%) bighorn sheep survived in 12 previous commingling experiments with domestic sheep, including only 1 of 26 in 4 experiments where presence of *M. ovipneumoniae* was reported or could be confirmed retrospectively (Foreyt and Jessup 1982; Foreyt 1989, 1990; Lawrence et al. 2010; Table S1, available online in Supporting Information).

Although Koch's second postulate (i.e., isolation of the agent in pure culture; Walker et al. 2006) has been repeatedly fulfilled, the ability of those cultures to reproduce the disease in healthy bighorn sheep (i.e., Koch's third postulate) is limited, perhaps because of virulence attenuation during cultural passage (Gilmour et al. 1979, Niang et al. 1998a, Besser et al. 2008). However, nasal washes from *M. ovipneumoniae*-colonized domestic sheep, treated to remove any detectable viable bacterial species other than *Mycoplasma*, do reproduce the disease in healthy bighorn sheep and the challenge strain of *M. ovipneumoniae* can be recovered from the pneumonic lungs of the affected animals, thereby fulfilling postulate 4 (i.e., re-isolation of the originally inoculated pathogen; Besser et al. 2014).

Many *Mycoplasma* spp. are host-specific, and the host range of *M. ovipneumoniae* is considered to be limited to Caprinae (Nicholas et al. 2008). Respiratory disease following infection with *M. ovipneumoniae* also has been reported in captive Dall's sheep and other wild Caprinae, including mountain goats (*Oreamnos americanus*), and muskox (*Ovibos moschatus*; Black et al. 1988, Handeland et al. 2014, Wolff et al. 2014). In a recent National Animal Health Monitoring System survey, Sheep 2011, the Animal and Plant Health

Inspection Service tested up to 16 adult females each in 453 randomly selected domestic sheep flocks from across the United States for *M. ovipneumoniae* nasal carriage and serum antibody. Most flocks (88%) tested positive for carriage (as determined by polymerase chain reaction [PCR] on nasal swabs). Larger operations were more likely to be PCR positive and all flocks with ≥ 500 adult females were PCR positive (USDA Aphis Veterinary Services 2015). Less extensive surveys of domestic goats reported 37.5–88% of flocks to be PCR positive on nasal swabs. Larger flocks were more likely to be positive for carriage (Heinse et al. 2016; Table S2, available online in Supporting Information). A host-specific pathogen commonly carried by domestic sheep and goats is consistent with the high mortality observed in captive bighorn sheep when commingled with domestic sheep but not when commingled with non-Caprinae livestock including cattle, horses, and llamas (Foreyt 1992, Foreyt and Lagerquist 1996, Besser et al. 2012a).

Additional evidence for *M. ovipneumoniae* as an epidemic agent is the transmission of 1 (or occasionally 2) multi-locus sequence types (strains) within an outbreak and a diversity of strains across outbreaks (Besser et al. 2012b, Cassirer et al. 2017). These strains of *M. ovipneumoniae* also link the all-age epizootics to the recurrent lamb pneumonia epizootics that follow (Cassirer et al. 2017). Strains detected in domestic sheep differ from those detected in domestic goats, suggesting host adaptation and coevolution within old world Caprinae (Maksimović et al. 2017). This divergence also provides a means for inferring the host species of origin.

CHARACTERISTICS OF RESPIRATORY DISEASE IN INDIVIDUALS

The diverse histopathologic lesions observed in experimental and naturally occurring bighorn sheep pneumonia, range from those typical of *Mycoplasma* infections (lymphocytic cuffing around airways and hypertrophy of the bronchial respiratory epithelium) to the often more dramatic and severe hemorrhagic, edematous, and necrotic lesions resulting from secondary bacterial infections (Miller 2001, Besser et al. 2008, Wood et al. 2017). This polymicrobial pneumonia is thought to occur when *M. ovipneumoniae* binds to and degrades the cilia of the trachea and bronchi, resulting in disruption of the mucociliary escalator (Niang et al. 1998c), the physiologic process for clearing bacteria from the lower respiratory tract. The impaired host immune defenses then allow inhaled opportunistic pathogens to establish multiple simultaneous infections of lung tissues with often fatal results.

The clinical course of bighorn sheep pneumonia may appear dramatic and short, but evidence from naturally occurring and experimental infection indicates that sub-clinical disease exists for several days to several weeks prior to development of obvious symptoms (Besser et al. 2008, Besser et al. 2014, Cassirer et al. 2017). This delay presumably represents the time required for *M. ovipneumoniae* to infect the airways and disrupt the mucociliary escalator. The latent period has important implications for management because animals might appear healthy for several weeks following

infection. By the time disease is evident, *M. ovipneumoniae* and other pneumonia pathogens already could be widespread in the population, even in individuals that still look healthy.

The original focus of *M. ovipneumoniae* research was infection in domestic sheep and goats where it is documented as an important, and probably under-diagnosed, cause of pneumonia in lambs and kids (Lin et al. 2008, Rifatbegović et al. 2011). Differences in the disease across host species suggest potential focal areas for research that may reveal why the disease is so devastating in wild sheep (Table 1). Higher nasal carriage rates ($\chi^2_1 = 35.49$, $P < 0.001$) and lower antibody prevalence in domestic sheep ($\chi^2_1 = 33.78$, $P < 0.001$; Table 1, Table S2) are consistent with an evolved tolerance of *M. ovipneumoniae*, defined as the ability to shed high levels of a pathogen with minimal morbidity or mortality (Råberg et al. 2009). Bighorn sheep resist infection and react to *M. ovipneumoniae* exposure with dramatic humoral immune responses, which could reduce carriage (Table 1), but also might trigger an auto-immune reaction. Such a reaction has been described in domestic lambs that develop respiratory disease associated with *M. ovipneumoniae* infection (Niang et al. 1998b). Robust bighorn sheep immune responses may also contribute to their disease.

Although *M. ovipneumoniae* may be associated with early pneumonia in domestic lambs (Bottinelli et al. 2017), juvenile domestic sheep are usually resistant to *M. ovipneumoniae* prior to weaning. Lambs born in persistently infected flocks often become infected during their third month of life (Table S3, available online in Supporting Information). Bighorn lambs are apparently completely susceptible to infection from birth (Besser et al. 2013), despite the similar magnitude and timing of passive transfer of maternal immunity in both species (Herndon et al. 2011, Highland 2016). Passively transferred bighorn sheep

antibodies might not protect from colonization or it could be that other forms of immunity are more important than the maternally transferred antibody-mediated immune response in defending the host from this pathogen (Plowright et al. 2013).

Domestic sheep herds usually harbor multiple strains of *M. ovipneumoniae* simultaneously (Thirkell et al. 1990; Ionas et al. 1991a, b; Parham et al. 2006). Therefore, intensive sampling and strain typing are required to confirm or rule out individual flocks as a source of *M. ovipneumoniae* transmission to bighorn sheep populations. In contrast, 1 or occasionally 2 strains appear to predominate in bighorn sheep populations (Cassirer et al. 2017). Immune response to *M. ovipneumoniae* is apparently strain-specific in both species, but disease outcomes of cross-strain infection are more severe in bighorn sheep (Alley et al. 1999, Felts et al. 2016, Justice-Allen et al. 2016, Cassirer et al. 2017).

PNEUMONIA IN BIGHORN SHEEP POPULATIONS

Die-Off Events

Many, if not most, bighorn sheep populations in the lower 48 states have endured all-age pneumonia die-offs (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012). These epizootics are the most obvious and dramatic manifestation of disease in bighorn sheep populations. During pneumonia outbreaks when animals are clinically ill, disease agents such as *M. ovipneumoniae* and Pasteurellaceae, usually transmitted through direct contact, may become airborne for short distances (Dixon et al. 2002, Besser et al. 2014). Pathogens can spread rapidly and expose nearly all individuals to infection (Bernatowicz et al. 2016, Ramsey et al. 2016, Cassirer et al. 2017). Severe, high mortality epizootics can ultimately cause extirpation or functional extinction of populations (Singer et al. 2000b); however, most pneumonia outbreaks do not kill entire populations. We estimated a median population decline of 48% (range = 5–100%) in 82 bighorn sheep disease events reported in 7 states and 2 provinces (Fig. 1, Table S4). Causes of the considerable divergence in mortality rates are not well understood but might be explained by heterogeneity in host immunity, pathogen virulence, and patterns of contact and transmission (Hobbs and Miller 1992).

We detected 28 different strains of *M. ovipneumoniae* in 45 bighorn sheep populations tested in 6 western states (Fig. 2B,C; Tables S5 and S6, available online in Supporting Information), each of which likely represents a separate spillover event that caused an all-age epizootic when first introduced. Domestic sheep and domestic goat *M. ovipneumoniae* lineages were both detected in bighorn sheep populations, but most strains detected in bighorn sheep fell within the domestic sheep clade (Kamath et al. 2016, Cassirer et al. 2017; Fig. 2C).

Clusters of the same strain in inter-connected populations, such as those along the border of Idaho, Oregon, and Washington in Hells Canyon, USA; in the Pancake Range

Table 1. Comparison of *M. ovipneumoniae* infection in domestic and bighorn sheep, USA, 1999–2016.

	Bighorn sheep	Domestic sheep
Infection outcome—naïve adults ^a	Bronchopneumonia	No disease
Infection outcome—lambs ^a	Bronchopneumonia 20–100% mortality	Coughing syndrome <2% mortality
Age of lambs at initial infection ^a	<1 week	Usually 8–12 weeks
Prevalence of carriage ^b	Low (median 22%)	High (median 56%)
Seroprevalence ^c	High (median 67%)	Low (median 30%)
Strain diversity within populations ^d	Usually 1	Usually many

^a Alley et al. (1999); Besser et al. (2008, 2014); Cassirer et al. (2013); USDA (2011).

^b Samples ($n = 1,267$) from 40 bighorn sheep populations in California, Idaho, Nevada, Oregon, Utah, and Washington and 47 domestic sheep flocks ($n = 2,508$ samples) in 13 states across the United States (USDA Aphis 2015).

^c Samples ($n = 1,589$) from 42 bighorn sheep populations in California, Idaho, Nevada, Oregon, Utah, and Washington and 37 domestic sheep flocks ($n = 323$ samples) across the United States (USDA Aphis 2015).

^d Parham et al. (2006), Cassirer et al. (2017).

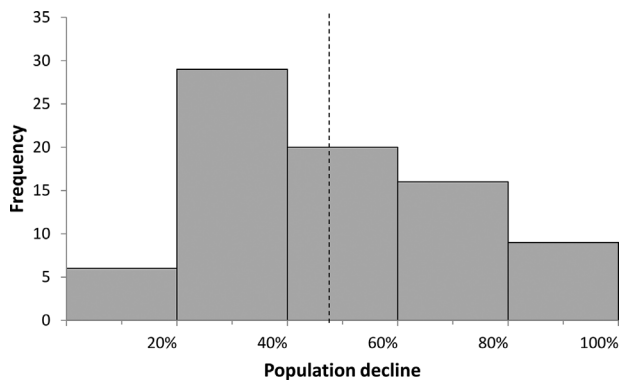


Figure 1. Population declines reported after pneumonia events in bighorn sheep populations in Alberta and British Columbia, Canada; and Idaho, Montana, Nebraska, Nevada, North Dakota, Oregon, Utah, and Washington, USA, 1978–2016. Dashed line represents median mortality of 48% in 82 pneumonia events. Data provided by state and provincial agencies and the Western Association of Fish and Wildlife Agencies Wild Sheep Working Group.

metapopulation of south-central Nevada, USA; and in the southern Nevada metapopulation (Fig. 2B), likely reflect a multiplier effect on a single spillover event when carrier bighorn sheep spread the pathogen across neighboring populations over time.

Multiple strains of *M. ovipneumoniae* observed within a single bighorn sheep population (Fig. 2B) often represent sequential pathogen invasion events. When a new strain is introduced into a population with ongoing infection, it may

replace the existing strain or eventually fade out. Retrospective analysis in the intensively sampled Hells Canyon metapopulation demonstrated a pattern of sequential spillovers and strain replacement or fadeout (Cassirer et al. 2017). Additional data and genomic analyses will be useful for confirming relationships among strains within and between populations and for more rigorous modeling of the ancestral phylogeny and transmission dynamics.

Pathogen Persistence

In 6 states (i.e., California, Idaho, Nevada, Oregon, Utah, and Washington), 63% of 155 populations where infection status is known, have been exposed to *M. ovipneumoniae*, including most native (never extirpated) herds (Fig. 2A). Exposure, as determined by the presence of *M. ovipneumoniae*-specific antibodies, indicates that at least some members of the population have been infected during their lifetime. Exposure does not confirm ongoing shedding, but infection is often maintained in exposed populations by (generally) asymptomatic carriers (Plowright et al. 2016, Cassirer et al. 2017).

Persistently infected populations have a high likelihood of prolonged periods of disease in juveniles and occasionally adults. High rates of pneumonia-induced lamb mortality (20–100%) between 4 and 14 weeks of age are common and reduce recruitment, limiting population growth or causing declines when combined with other mortality factors (Ryder et al. 1992, Enk et al. 2001, Smith et al. 2014, Smith et al. 2015). Some populations rebound (Coggins and Matthews

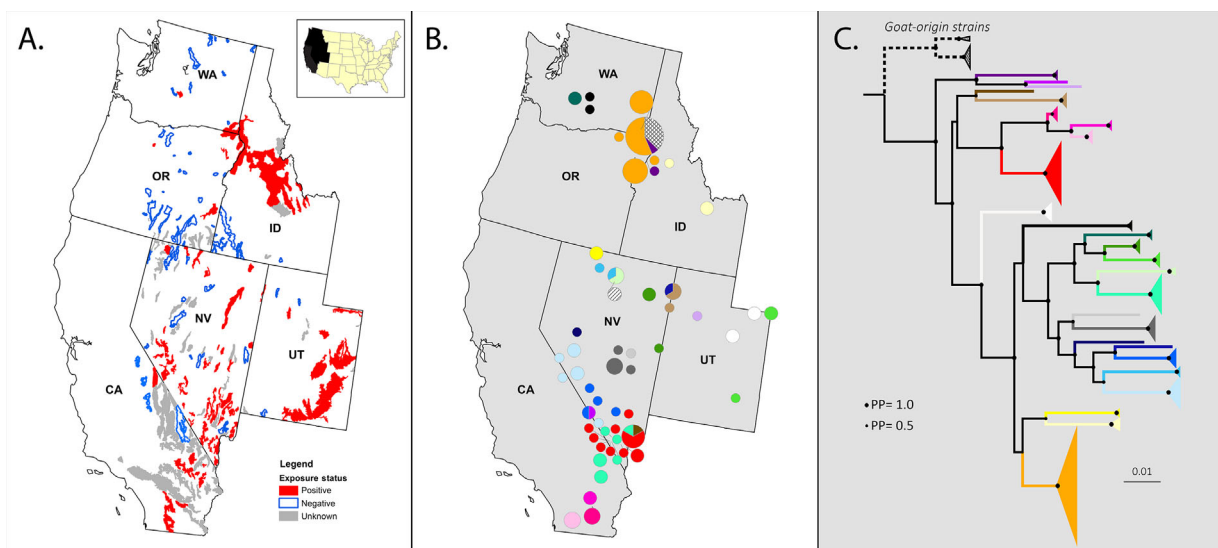


Figure 2. Exposure status and strain types of *Mycoplasma ovipneumoniae* detected in bighorn sheep populations in Washington, Oregon, Idaho, Utah, California, and Nevada, USA. A) Exposure of bighorn sheep populations to *M. ovipneumoniae*, 1999–2016. We assigned exposure based on results of blood serum samples ($n \geq 10$ from each population) submitted to the Washington Animal Disease and Diagnostic Laboratory for *M. ovipneumoniae*-specific serum antibody testing. Populations were classified as positive if antibodies were detected in at least one sample. Populations were classified as negative if no antibodies were detected from ≥ 15 samples. B) Spatial distribution of 28 *M. ovipneumoniae* genotypes (strains) obtained from bighorn sheep, 1984–2016. Each strain type is identified by a different color (domestic sheep origin, $n = 26$) or hatching (domestic goat origin, $n = 2$). Strains of the same color represent well-supported monophyletic clusters (posterior probability, PP > 0.95) with 99.7% to 100% sequence identity (as in panel C). In general, strains that are shades of the same colors are more closely related than strains of different colors. Pie charts indicate the proportional composition of strain types found at a given location over the entire time period of sampling. Chart size is relative to sample size. C) Bayesian majority rule consensus phylogeny of *M. ovipneumoniae* strains derived from bighorn sheep, with colors corresponding to strains shown in panel B. We based phylogenetic analyses on multilocus sequence typing data from 4 genetic loci (16S, IGS, *rpoB*, *gyrB*). Node support is reported as PP, with circle size relative to value (only PP > 0.60 shown). Scale bar indicates genetic distance in units of nucleotide substitutions per site. Dashed line represents the *M. ovipneumoniae* lineage derived from domestic goats.

1992, Jorgenson et al. 1997), but in others there is no trend towards recovery for decades (Manlove et al. 2016). In these cases, the demographic costs of pathogen persistence can outweigh the effects of the initial epizootic. Persistently infected populations also pose a disease risk for adjacent herds and, if they are used as source stock for translocations, moving carriers can inadvertently spread infection over long distances.

Chronically infected populations occasionally experience years with no evidence of disease in juveniles or adults. In approximately 20% of years following all-age disease epizootics in Hells Canyon, lamb survival was high and similar to that observed in unexposed populations (Cassirer et al. 2013). These sporadic disease fadeouts may be due to a delay or failure of *M. ovipneumoniae* transmission to susceptible lambs, as opposed to local pathogen extirpation, because pneumonia epizootics recur in subsequent years. A single or even several years with apparently healthy rates of lamb survival is not necessarily a harbinger of pathogen fadeout and population recovery (Manlove et al. 2016).

Social behavior likely plays an important role in determining the patterns of pneumonia epizootics and disease fadeout. Males are more likely to be directly associated with spillover and spread within and across populations during all-age outbreaks simply because they have larger home ranges and make more long distance movements (DeCesare and Pletscher 2006, O'Brien et al. 2014, Borg et al. 2016). However, dam-lamb and lamb-lamb interactions may be the most important routes of transmission in persistently infected populations (Manlove et al. 2017). Population substructure seems to protect some nursery groups from pathogens (Manlove et al. 2014) perhaps because no carrier dams are present. However, substructuring also might decouple contact rates and associated pathogen transmission from population size. If contact rates remain high as populations decline, transmission may never drop below the threshold required for pathogen extinction. This form of frequency-dependent transmission is common in social animals, and allows disease to persist at low population sizes. This can ultimately lead to host extirpation especially when combined with other stochastic events affecting small populations (de Castro and Bolker 2005).

MONITORING POPULATIONS FOR INFECTION AND DISEASE

All-age pneumonia epizootics are usually readily detected by observations of sick and dying sheep where populations are being actively monitored or are easily observed. However, low mortality outbreaks and epizootics in small and remote populations may be overlooked and underreported. Bighorn sheep also die from other diseases and not all sheep with clinical signs of respiratory disease (for example coughing) have pneumonia. Necropsy and laboratory testing are recommended when animals die from unknown causes, or when pneumonia is suspected. Pneumonia epizootics should be considered as a plausible cause when there is a sudden decline in a bighorn sheep population, particularly if followed by low recruitment.

Outside of all-age outbreaks, juvenile survival, particularly during the first 4 months of life, is the best demographic indicator of health status in bighorn sheep populations. Poor survival to weaning, (~4 months of age; Festa-Bianchet 1988), is the most sensitive signal of pneumonia-induced mortality in lambs. In Hells Canyon, there was a 100% probability of pneumonia being detected when survival to weaning was <50% (Cassirer et al. 2013, Manlove et al. 2016; Fig. 3A); however, this relationship might differ in areas with higher rates of non-disease-related neonatal mortality. Recruitment of juveniles as yearlings and population trend are less clear and specific metrics for classification of health status. Although most populations are stable or decline slowly during periods of persistent infection, pneumonia also might be present when lamb:female ratios at 9–12 months (recruitment) are ≤ 0.30 (Fig. 3A), even if populations are stable or slightly increasing (Fig. 3B, Table S7 available online in Supporting Information).

Diagnostic testing procedures for respiratory disease are continually changing as technology advances and knowledge of the disease and disease agents evolve. Comprehensive testing guidelines for wild sheep produced by the Western Association of Fish and Wildlife Agencies Wildlife Health Committee (2015) provide a good recent overview for a broad array of pathogens. Sampling for *M. ovipneumoniae* should be a part of any bighorn sheep health surveillance protocol and can also be used to monitor potential sources of domestic spillover. The most efficient diagnostic strategies for detection vary by host species and by infection stage. In acute infection (e.g., during all-age or lamb pneumonia epizootics in bighorn sheep, or in 8–16-week-old domestic lambs or kids in an enzootic flock), *M. ovipneumoniae* can be detected by PCR tests in a high proportion of animals' nasal swabs or pneumonic lung tissues. Infection status of domestic sheep and goat flocks is also best determined by PCR tests on nasal swabs. Given the high *M. ovipneumoniae* shedding prevalence in domestic sheep flocks (median 0.56; Table 1), PCR testing on swab samples from 10 adults should be sufficient (99% probability, binomial test) to detect whether *M. ovipneumoniae* is present. Repeated sampling is recommended to confirm negative status. In contrast, determining the exposure status of chronically infected bighorn sheep herds is most efficiently done by testing for serum antibodies, given the relatively high seroprevalence (median 0.67) and lower PCR prevalence in wild sheep (Table 1). Blood serum samples from 15 animals are generally adequate to determine exposure status if prevalence is ≥ 0.25 (99% probability, binomial test). If no antibodies are detected, the population can be considered unexposed, unless samples are collected recently after transmission, prior to immune response development. Nasal swabs from ≥ 18 animals should be adequate (85% probability) to detect shedding by PCR in bighorn sheep populations with *M. ovipneumoniae* prevalence of ≥ 0.10 . Larger sample sizes may be required to account for non-detection error associated with field sampling and diagnostic testing (Walsh et al. 2016). Strain-type can be identified in PCR-positive samples. Nasal or sinus swabs can also be collected from

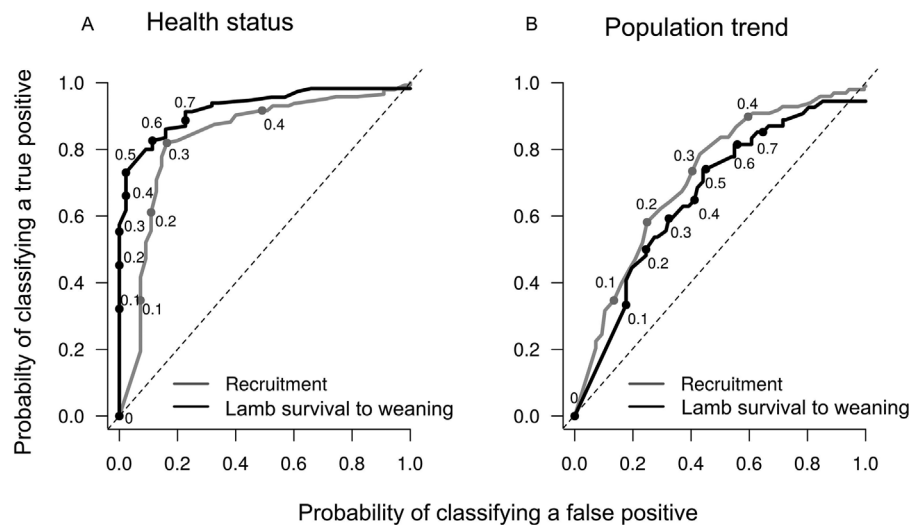


Figure 3. Receiver operating characteristic (ROC) curves identifying optimal lamb survival to weaning (black) and recruitment lamb:female ratios (gray) cut-off values for correctly classifying A) presence of pneumonia and B) declining population trend in bighorn sheep populations in Hells Canyon, Idaho, Oregon, and Washington, USA, 1997–2015 (Manlove et al. 2016). The y axis indicates sensitivity and the x axis indicates inverse specificity of lamb survival and recruitment values. A perfect predictor would have a value of 1 on the y axis and 0 on the x axis for a score of 1 AUC (area under curve). The dashed line represents values with no ability to predict categories (0.50 AUC). A) Lamb survival to weaning (AUC = 0.93) was an excellent predictor of health status and performed better than recruitment (AUC = 0.84). B) Recruitment (AUC = 0.72) was a better predictor of population trend than juvenile survival to weaning (AUC = 0.66). Optimal cut-off values for assigning presence of pneumonia were juvenile survival to weaning of <0.60 (84% accuracy) or recruitment of <0.30 lambs:female (82% accuracy). The optimal cut-off value for classifying populations as declining was recruitment of <0.20 lambs:female (69% accuracy).

fresh or frozen dead animals including heads of hunter-harvested bighorn sheep. Formalin-fixed paraffin-embedded pneumonic lung tissue blocks routinely archived by most diagnostic laboratories for histopathology also provide a DNA source for investigating historical presence and strain types of *M. ovipneumoniae* and other pathogens.

MANAGEMENT OF PNEUMONIA IN BIGHORN SHEEP

Wildlife managers and veterinarians have tried many techniques for controlling and mitigating respiratory disease in wild sheep populations, including administering antibiotics (Coggin 1988, Rudolph et al. 2007, McAdoo et al. 2010), vaccination (Cassirer et al. 2001, Sirochman et al. 2012), mineral supplementation (Coggin 2006, Sirochman et al. 2012), anthelmintic treatment (Miller et al. 2000, Goldstein et al. 2005), selective culling (Edwards et al. 2010, Bernatowicz et al. 2016, Ramsey et al. 2016), partial or complete depopulation (Cassirer et al. 1996, McFarlane and Aoude 2010, Bernatowicz et al. 2016), augmentation, and limiting population size and dispersal. The variety of methods employed, and the lack of clear successes, partially reflects past uncertainty over the causative agents and biological processes involved. The *ad hoc* nature of some of the treatments also limits broader inference. More rigorous testing of a broad ensemble of approaches for management is needed to account for the inherent challenges and variability associated with managing disease in free-ranging wildlife.

Large-scale pathogen eradication is rarely seen as a realistic goal (Klepac et al. 2013), particularly in the presence of a reservoir host, and indeed is not considered a viable option for wild sheep respiratory disease. Instead, more practical

management objectives include controlling the spatial extent or prevalence of the pathogen, facilitating natural pathogen extinction, or reducing the demographic costs of infection (Wobeser 2002, Joseph et al. 2013). Attempts to manage bighorn sheep pneumonia fall broadly into 2 categories: 1) strategies that directly aim to reduce exposure and transmission such as preventing spillover, treatment with antibiotics, vaccination, targeted culling of shedders, reducing population size or density, and population eradication; and 2) strategies that aim to increase individual resistance or herd resilience, including improving nutritional condition, increasing genetic diversity, managing co-infection, or increasing or modifying spatial structuring. Some approaches (such as vaccination or density reduction) could conceivably have application in both categories.

Preventing exposure, theoretically and in practice, offers the most direct and effective method for disease control. Managing transmission is a component of disease prevention strategies for most zoonoses and other spillover diseases (Ebinger et al. 2011, Viana et al. 2014) including test and cull for brucellosis, oral vaccination for rabies, and reduction of deer density for tuberculosis (Rupprecht et al. 1986, Schmitt et al. 2002, Slate et al. 2005, Schumaker et al. 2012). However, managing transmission can be a long-term and costly endeavor. Promoting individual resistance and population resilience has theoretical and empirical support in a number of systems. In general, managing populations to maximize their individual- or herd-level resilience makes good sense (Stephen 2014). Whether increased resistance and resilience can offset the costs of an exotic pathogen like *M. ovipneumoniae*, which generally produces high mortality rates in non-adapted but otherwise robust hosts, remains to

be seen. Below, we discuss past performance and future potential of these management strategies in combating bighorn sheep pneumonia.

Preventing Spillover and Pathogen Invasion

State and federal natural resource agencies have widely instituted policies to prevent pathogen spillover by encouraging or requiring spatial separation between wild sheep and domestic sheep and goats (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012, Bureau of Land Management 2016). Federal and state policies are informed by models, such as the USDA Forest Service's Bighorn Sheep Risk of Contact Tool (Woolever et al. 2015), which incorporate bighorn sheep space use, habitat preferences, foray probabilities, and demographics (Clifford et al. 2009, Cahn et al. 2011, Carpenter et al. 2014, O'Brien et al. 2014) to identify geographic locations with high risk of domestic-wild sheep contact. These models allow comparison of proposed management alternatives and assessment of population-level consequences for bighorn sheep. Resulting actions may take the form of closing or retiring public grazing allotments, altering their timing of use, trucking rather than trailing sheep between pastures, or changing grazing classification from domestic sheep or goats to other livestock (USDA Forest Service 2010, Bureau of Land Management 2017).

Other preventive management practices include capturing or culling escaped domestic sheep on bighorn sheep ranges and removing wild sheep observed near or commingling with domestics. Outreach efforts on private and public lands have encouraged landowner or public lands grazing permittee cooperation in double-fencing domestic sheep flocks in wild sheep habitat, using additional guard dogs, penning domestic sheep and goats at night, not turning sick sheep out to pasture, counting domestic sheep more frequently to better detect and gather strays, notifying local wildlife officials if wild sheep are observed near domestic sheep, and encouraging use of other best management practices (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012).

We are unaware of any formal evaluation of the success of existing separation strategies in preventing new outbreaks, though the regular appearance of new *M. ovipneumoniae* strains in bighorn sheep herds suggests there is room for improvement. Nevertheless, cross-species contact mitigation efforts almost certainly play a crucial role in reducing pathogen invasion. More work is needed to assess the strengths and weaknesses of existing approaches and to devise new and better strategies for managing both domestic and wild sheep to reduce transmission risk. Efforts are currently underway to investigate the feasibility of developing and maintaining *M. ovipneumoniae*-free domestic flocks, which could help reduce the significant risk of pathogen transmission from small domestic sheep and goat herds on private lands (Sells et al. 2015, Heinse et al. 2016, Cassirer et al. 2017).

Another paradigmatic approach to preventing pathogen introduction is reducing density of wild sheep populations. Associative studies (Monello et al. 2001, Sells et al. 2015)

report a positive relationship between wild sheep relative density (or population size) and risk of respiratory disease outbreaks. This relationship could mechanistically result from larger or higher density populations occupying a greater area and dispersing more widely or more often than smaller, lower density herds, with the consequence that increased density corresponds to increased contacts with neighboring domestic sheep or infected wild sheep herds (Monello et al. 2001). Evidence, however, for a density-dependent relationship in movements and dispersal in ungulates is limited and equivocal (Loison et al. 1999, Long et al. 2008) and pre-outbreak population sizes are often small (<50 to 200 animals) and do not differ from sizes of populations that remain healthy (Monello et al. 2001, Shannon et al. 2014).

Many reintroduced bighorn sheep populations experience robust or even exponential growth following initial establishment. When these populations are exposed to respiratory pathogens they often undergo die-offs followed by a prolonged period of low lamb recruitment, limiting recovery (Manlove et al. 2016). As a result, populations are often largest just prior to outbreaks, leading to a statistical, but not necessarily biological, association between population size and outbreak risk. The expected biological processes underlying a presumed density-dependent relationship are not evident, such as declining population growth rate or reduced juvenile recruitment (Jorgenson et al. 1997, Monello et al. 2001). Therefore, it is unknown whether reducing populations or keeping them small would actually mitigate risk, or whether disease outbreaks are simply associated with healthy, growing, and susceptible populations. Future work could pursue the underlying mechanisms directly and experimentally. Understanding the nature of observed associations of pneumonia and population size in bighorn sheep is needed to help minimize disease risk and maximize the number and distribution of wild sheep on the landscape.

Translocations have been widely and successfully used to increase the numbers, distribution, and genetic diversity of bighorn sheep populations (Singer et al. 2000a, Hogg et al. 2006, Olson et al. 2012). Translocations also present a clear risk for anthropogenically assisted pathogen introductions and opportunities for exposure at release sites (Cunningham 1996, Deem et al. 2001, Sainsbury and Vaughn-Higgins 2012, Aiello et al. 2014). Moving animals known to be positive for pneumonia pathogens into new ranges is risky (Western Association of Fish and Wildlife Agencies Wildlife Health Committee 2015). Mixing bighorn sheep from populations known to harbor pathogens with naïve animals, can and has, had poor results (Sandoval et al. 1987). Even if a pathogen is present in both source and recipient populations, immunity may not provide universal protection (Dassanayake et al. 2009, Cassirer et al. 2017). Most state, federal, and provincial agencies use health screenings to inform wild sheep translocation decisions. Careful matching of pathogen profiles, including relevant bacteria, viruses, and parasites in source, recipient, and adjacent bighorn sheep populations and selecting release sites with low risk of contact with domestic sheep and goats are important for translocation success. In practice, health surveys may be

conducted a year in advance at the herd level and with imperfect pathogen detection probabilities, resulting in uncertainty surrounding an individual's health status at the time of translocation. Furthermore, *M. ovipneumoniae* strain typing would not be expected to detect possible epitope variation resulting in immune escape, and health screenings are only as good as our knowledge of what to look for. Improved molecular-based approaches for detecting and describing pathogens and their associated virulence factors are needed. Development of rapid animal-side tests is in progress and, if successful, could also contribute to reducing disease risks posed by translocations.

Reducing Transmission During and Post-Epizootics

A number of agencies have attempted to manage active respiratory disease outbreaks. However, no management action, absent population eradication, has successfully stopped a pneumonia outbreak, and there is no evidence that any intervention has consistently reduced morbidity, mortality, or spread of disease. In part, this is due to the unplanned nature of outbreaks and the inability to randomly assign treatments and controls to matched populations to reliably test for an effect. Nevertheless, efforts to halt epizootics by administering antibiotic treatments (Sandoval et al. 1987, Coggins 1988, Rudolph et al. 2007, McAdoo et al. 2010), and by conducting random and selective culls (Cassirer et al. 1996, Edwards et al. 2010, Bernatowicz et al. 2016, Ramsey et al. 2016) have generally had mixed or negative results. In other wildlife species, depopulation has been successfully employed to prevent spread between populations, but culling zones or population segments to stop the spatial spread of epidemics have met with limited success (Wobeser 2002). Culling is rarely successful because by the time an epidemic is detectable, transmission is usually well under way; even if culling slows transmission, it is unlikely to stop it given imperfect detection of symptomatic animals, long infectious periods, ongoing contacts, and undetected animal movements within and between populations. Lack of success with antibiotics and vaccines administered during or after outbreaks may be a function of their low efficacy, targeting the wrong agent, or an inability to administer them appropriately in most free-ranging bighorn sheep populations.

Depopulation and reintroduction has occasionally been used in an attempt to manage small, particularly poorly performing herds struggling with persistent disease. Although this method may be effective when all members of the former herd are removed, significant effort is needed to ensure complete removal and that the ongoing risk of pathogen introduction is low. A current experimental management effort offering an alternative to depopulation of persistently affected populations exploits the relatively low shedding prevalence of *M. ovipneumoniae* in bighorn sheep by removing only chronic carrier females (Bernatowicz et al. 2016). The goal of this experiment is to stop the chain of transmission from dams to lambs and facilitate pathogen fade-out. If successful, this technique may be best applied to small, accessible populations, where extensive testing is

feasible, and the stochastic mortality of chronic carriers may bolster an active selective removal. In general, test-and-cull success hinges on test sensitivity, animal handling opportunity, pathogen prevalence, and the duration over which management is implemented. Targeted removal works best when a few individuals are responsible for most of the transmission (Lloyd-Smith et al. 2005, Streicker et al. 2013) and may require complete eradication of these carriers. For example, although test-and-cull efforts to control brucellosis in elk (*Cervus canadensis*) successfully met goals of reducing local prevalence (Scurlock et al. 2010, Schumaker et al. 2012) they never eradicated the disease, and upon the program's cessation, prevalence rapidly increased (Wyoming Game and Fish Department 2016). Test-and-cull strategies would ideally be timed to coincide with the lowest-possible pathogen prevalence and the highest levels of immunity, although we currently do not know when those minima and maxima occur.

Managing for Resistance and Resilience

Managing disease by maximizing individual resistance or population resilience has received renewed interest, particularly in the face of continuing challenges associated with direct control of transmission. Theoretical and empirical work across humans, domestic animals, and wildlife suggests that manipulating physiological condition, genetics, or co-infection can alter rates of morbidity and mortality and reduce infection intensity, which may in turn feedback on population-level dynamics (Beldomenico and Begon 2010). However, several studies have found that tradeoffs often exist between enhancing disease resistance and controlling transmission. For example, increasing food supply can minimize parasite-induced mortality (Pedersen and Greives 2008) but may also facilitate transmission (Becker et al. 2015), managing co-infections can reduce morbidity and mortality but can also accelerate pathogen spread (Ezenwa and Jolles 2015), and metapopulation structure can enhance disease spread while simultaneously allowing higher numbers of hosts to survive (Hess 1996, McCallum and Dobson 2002).

There are numerous examples of management actions intended to bolster individual resistance and overall population performance in struggling bighorn sheep populations but little systematic evaluation as to their efficacy. For example, there is no clear evidence of a causal relationship between nutritional condition and susceptibility to respiratory disease in bighorn sheep. Certainly the many experiments in captivity show that optimally provisioned bighorn sheep still succumb at high rates upon exposure to respiratory pathogens. Disease resistance may be correlated with genetic diversity (Luikart et al. 2008, Savage and Zamudio 2011) and researchers continue to seek evidence of host genetic resistance to respiratory disease, which might be expected in herds that are demographically successful even in the presence of long-term pathogen persistence but, to date, a genetic basis has not been found for the susceptibility of wild sheep to pneumonia (Gutierrez-Espeleta et al. 2001, Boyce et al. 2011). Currently, multi-jurisdictional efforts are

underway to collect data on animal condition, genetics, and pathogens to better understand their interactions with wild sheep health.

At the population level, maximizing resilience might include promoting large, widely distributed, genetically diverse metapopulations with spatial structuring and a range of behaviors (de Castro and Bolker 2005, Hess 1996). Indeed, there is some evidence that larger wild sheep populations may experience lower rates of mortality during pneumonia epizootics and are more able to recover than their smaller counterparts (Singer et al. 2001, Cassaigne et al. 2010). Furthermore, increasing population substructure may create asynchrony in transmission across groups of animals. Although this may not prevent epizootics and could actually increase pathogen persistence at the herd or metapopulation level (Grenfell and Harwood 1997, Swinton et al. 1998, Park et al. 2002), it might buffer against simultaneous population-wide epizootics and facilitate stochastic pathogen extinction from sub-herd or population segments (Cross et al. 2005). More work is needed to determine whether or not spatial structuring shields bighorn sheep populations from the worst outcomes of disease and how population structure might affect disease persistence. Current efforts are underway on a limited basis to expedite formation of metapopulation structure by assisted colonization of adjacent range. These manipulations may be most applicable to large, healthy populations.

MANAGEMENT IMPLICATIONS

The extensive costs of pathogen introduction and transmission observed across a wide range of habitats and populations indicate that preventing spillover is the most pressing immediate priority for management of pneumonia in bighorn sheep. Collaboration by wildlife and livestock managers on research and in practice is needed to develop more effective, sustainable approaches to reduce ongoing pathogen transmission from domestic small ruminants to wild sheep. Transmission risks posed by moving bighorn sheep to expand populations are also recognized and should be mitigated before translocations are conducted. In the absence of spillover, selection on the host and the pathogen may eventually lead to a less destructive relationship between wild sheep and the bacteria involved in pneumonia. However, considerable theory suggests that evolution toward increased resistance or reduced virulence is not always expected (Alizon et al. 2009, Osnas et al. 2015). Effective tools are needed to actively restore persistently infected stagnant or declining populations. A comprehensive examination of disease dynamics across populations to better understand how recovery occurs naturally would be useful to inform management of pneumonia in exposed populations.

In the long-term, agencies will need better strategies for the management of larger interconnected bighorn sheep populations for species viability. Engaging a diversity of perspectives in the wildlife, domestic animal, and health sciences through an inter- or trans-disciplinary process could provide new directions or refine existing approaches for management of healthy, resilient populations (Choi and Pak 2007, Allen-Scott et al. 2015). Natural

experiments and designed experiments conducted in an adaptive management framework can also accelerate learning about complex natural systems (Walters and Green 1997, Craig et al. 2012, Williams and Brown 2016). Inter-jurisdictional collaboration can greatly facilitate and, in many cases, is required for successful adaptive management. Replicated interventions with clear hypotheses, objectives, and defined expected outcomes accompanied by monitoring of treatments and controls could greatly advance understanding in the face of uncertainty and speed progress towards developing successful strategies for managing pneumonia in wild sheep.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

Risk of Pathogen Spillover to Bighorn Sheep from Domestic Sheep and Goat Flocks on Private Land

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ABSTRACT Bighorn sheep (*Ovis canadensis*) across North America have suffered population losses due to polymicrobial pneumonia typically initiated by spillover events of bacteria from domestic sheep and goats. Because vaccination or treatment of individual animals remains an elusive goal and pneumonia often persists in bighorn herds for years or decades following infection, preventing contact between domestic and wild animals is widely accepted as the best prophylactic. For the past decade, most management efforts have focused on the risks associated with commercial sheep grazing on public lands; less attention has been paid to risks to bighorns from small flocks of domestic sheep and goats managed entirely on private land. We surveyed owners of 40 sheep or goat flocks located near bighorn sheep herds in central and southeastern Washington, USA during 2014 and 2015, to better understand their knowledge level, management practices, and willingness to reduce risks. Over one-third of sheep or goat owners had no knowledge of the potential for pathogen spillover to bighorns, but all were interested in reducing risk of interacting with bighorns, particularly by options that did not restrict their autonomy. We also sampled nasal mucosae of 137 animals in 24 flocks for presence of *Mycoplasma ovipneumoniae*, the bacterium most closely associated with bighorn pneumonia. *M. ovipneumoniae* was detected in 37.5% of sheep or goat flocks sampled, and animals had escaped their enclosures in 78% of these. Physical contact, and thus pathogen spillover from domestic sheep or goats living in small, private flocks in close proximity to bighorns is clearly a risk. We provide recommendations to agency staff on identifying, prioritizing and testing small herds, and then working with owners to reduce the risk of pathogen spillover.

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KEY WORDS bighorn sheep, disease, domestic goats, domestic sheep, landowner attitudes, *Mycoplasma ovipneumoniae*, *Ovis canadensis*, pneumonia.

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Evidence for strain-specific immunity to pneumonia in bighorn sheep

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Evidence for Strain-Specific Immunity to Pneumonia in Bighorn Sheep

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ABSTRACT Transmission of pathogens commonly carried by domestic sheep and goats poses a serious threat to bighorn sheep (*Ovis canadensis*) populations. All-age pneumonia die-offs usually ensue, followed by asymptomatic carriage of *Mycoplasma ovipneumoniae* by some of the survivors. Lambs born into these chronically infected populations often succumb to pneumonia, but adults are usually healthy. Surprisingly, we found that introduction of a new genotype (strain) of *M. ovipneumoniae* into a chronically infected bighorn sheep population in the Hells Canyon region of Washington and Oregon was accompanied by adult morbidity (100%) and pneumonia-induced mortality (33%) similar to that reported in epizootics following exposure of naïve bighorn sheep. This suggests an immune mismatch occurred that led to ineffective cross-strain protection. To understand the broader context surrounding this event, we conducted a retrospective analysis of *M. ovipneumoniae* strains detected in 14 interconnected populations in Hells Canyon over nearly 3 decades. We used multi-locus sequence typing of DNA extracts from 123 upper respiratory tract and fresh, frozen, and formalin-fixed lung samples to identify 5 distinct strains of *M. ovipneumoniae* associated with all-age disease outbreaks between 1986 and 2014, a pattern consistent with repeated transmission events (spillover) from reservoir hosts. Phylogenetic analysis showed that the strain associated with the outbreak observed in this study was likely of domestic goat origin, whereas strains from other recent disease outbreaks probably originated in domestic sheep. Some strains persisted and spread across populations, whereas others faded out or were replaced. Lack of cross-strain immunity in the face of recurrent spillovers from reservoir hosts may account for a significant proportion of the disease outbreaks in bighorn sheep that continue to happen regularly despite a century of exposure to domestic sheep and goats. Strain-specific immunity could also complicate efforts to develop vaccines. The results of our study support existing management direction to prevent contacts that could lead to pathogen transmission from domestic small ruminants to wild sheep, even if the wild sheep have previously been exposed. Our data also show that under current management, spillover is continuing to occur, suggesting that enhanced efforts are indicated to avoid introducing new strains of *M. ovipneumoniae* into wild sheep populations. We recommend looking for new management approaches, such as clearing *M. ovipneumoniae* infection from domestic animal reservoirs in bighorn sheep range, and placing greater emphasis on existing strategies to elicit more active cooperation by the public and to increase vigilance on the part of resource managers. © 2016 The Wildlife Society.

KEY WORDS bighorn sheep, disease ecology, Hells Canyon, livestock-wildlife interface, molecular epidemiology, multi-locus sequence typing, *Mycoplasma ovipneumoniae*, *Ovis canadensis*.

Pneumonia in bighorn sheep (*Ovis canadensis*) is a population-limiting disease associated with transmission of pathogens from domestic sheep and goats (Foreyt and Jessup 1982, Besser et al. 2013, Cassirer et al. 2013). As with many other wildlife diseases, a lack of comprehensive, system-specific information hampers disease management (Besser

et al. 2013, Joseph et al. 2013). Limiting contact with domestic sheep and goats is the primary strategy for preventing disease emergence in bighorn sheep (Western Association of Fish and Wildlife Agencies Wild Sheep Working Group 2012, The Wildlife Society 2015). Although currently the best management option available, implementation is politically and logistically difficult because of the natural movements of wild animals, unregulated presence of domestic sheep and goats on private lands, and concerns by the livestock industry about losing access to public grazing allotments. Reliable knowledge about health

threats and potential solutions will help wildlife and land managers make appropriate risk management decisions that will succeed in resolving the problem of pneumonia in bighorn sheep.

Based on the hypothesis that *Mannheimia haemolytica* expressing leukotoxin is the key causal pathogen, researchers have tested numerous vaccines to boost immunity to disease in bighorn sheep. However, so far no vaccine has protected wild sheep commingled with domestic sheep or goats in captive settings or shown potential for efficacy in free-ranging animals (Callan et al. 1991, Kraabel et al. 1998, Cassirer et al. 2001, Subramaniam et al. 2011, Sirochman et al. 2012). There may be several reasons for the elusiveness of an effective vaccine. First, there is a basic question as to the role of *M. haemolytica* in the disease (Besser et al. 2013) and second there are significant technical difficulties associated with vaccine development and application. Experimental challenge with leukotoxin-positive *M. haemolytica*, a well-described respiratory pathogen in domestic ruminants, is lethal to bighorn sheep in captivity (Foreyt et al. 1994, Dassanayake et al. 2009). However, *M. haemolytica* is only weakly associated with pneumonia epizootics in free-ranging bighorn sheep populations (Besser et al. 2012b). The pathology, microbiology, and the course of disease experimentally induced with *M. haemolytica* also do not match observations from the field (Besser et al. 2014). Recently, application of sensitive molecular diagnostic techniques on high quality samples led to identification of *Mycoplasma ovipneumoniae*, a previously overlooked bacterium, as the pathogen most strongly supported as a primary causal agent of pneumonia in bighorn sheep (Besser et al. 2008, 2012a, b).

M. ovipneumoniae is host-specific to Caprinae, and is frequently carried asymptotically by domestic sheep and goats (Martin and Aitken 2000). When introduced into naïve bighorn sheep populations, outbreaks of polymicrobial pneumonia ensue, sometimes resulting in high mortality in all age classes (Besser et al. 2008, 2014). After all-age pneumonia outbreaks, surviving adults usually maintain good health and normal life spans, although some individuals chronically carry *M. ovipneumoniae* in their upper respiratory tract (Besser et al. 2013). Both carriers and non-carriers are resistant to disease although this protection fails to prevent epizootics in lambs (Plowright et al. 2013, Manlove et al. 2016).

M. ovipneumoniae is also associated with mild and transient respiratory disease, usually in juveniles, in its normal domestic sheep and goat hosts (DaMassa et al. 1992, Martin and Aitken 2000). However, several investigators have reported that *M. ovipneumoniae* infections in domestic sheep and goats can cause severe pneumonia, particularly when multiple strains are present (Parham et al. 2006, Rifatbegović et al. 2011). This could be linked to a strain-specific immune response that fails to provide universal protection. Many pathogens are able to evade host immune responses by expressing a diversity of surface-exposed targets for neutralizing antibodies. From influenza virus to *Mycoplasma* spp., antigenic variation within and across strains enables

immune escape by pathogens and also complicates development of vaccines (Citti et al. 2010, Vink et al. 2012, Quiñones-Parra et al. 2014).

We documented the effects of invasion of a novel strain of *M. ovipneumoniae* into a group of free-ranging bighorn sheep that had harbored adult carriers for nearly 20 years following an all-age pneumonia outbreak (Cassirer et al. 1996, Plowright et al. 2013). Our expectation was that these adults were immune to the pathogen and that invasion of a new strain would not cause disease.

STUDY AREA

We conducted this study near Heller Bar at the mouth of the Grande Ronde River in Asotin County, Washington, USA (46.079° N, -116.986° W). The area was located in low elevation (250–1,250 m) canyon grasslands and cliffs along the breaks of the Grande Ronde and Snake Rivers on the northern edge of Hells Canyon. Summers were hot ($\bar{x}$ highs in Jul and Aug = 26–32° C) and winters were mild ($\bar{x}$ lows in Dec and Jan = -2 to 2° C). Average annual precipitation was 31 cm. July and August were the driest months and peak precipitation occurred in May. Plant associations were dominated by perennial bunchgrass (*Pseudoroegneria spicata* and *Festuca idahoensis*) communities, with deciduous riparian shrub stringers and upland shrub-fields. Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*) stands occurred on northerly aspects. In addition to bighorn sheep, common ungulates in the study area included mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and elk (*Cervus elaphus*). Potential predators of bighorn sheep included cougars (*Felis concolor*), bobcats (*Lynx rufus*), coyotes (*Canis latrans*), wolves (*C. lupus*), and black bears (*Ursus americanus*). Over 50% of the area was publicly owned and managed by federal and state agencies chiefly for wildlife, recreation, and seasonal (spring) cattle grazing. A low density, unincorporated rural community was scattered on adjoining private rangelands at the mouth of the Grande Ronde River and along the adjacent Snake River.

Following extirpation in the early 1900s, the Washington Department of Fish and Wildlife reintroduced bighorn sheep to the Joseph Creek Wildlife Area near Heller Bar. Between 1977 and 1989, 39 sheep were translocated from Washington, Oregon, and Montana to establish the Black Butte population (Johnson 1995). This became one of 16 interconnected populations that comprise the Hells Canyon bighorn sheep metapopulation. The Black Butte population increased to approximately 215 animals before a pneumonia outbreak occurred in 1995; 70% of the sheep died or were transferred to captivity in an attempt to stop the epidemic (Cassirer et al. 1996). The source of the outbreak was thought to be domestic sheep or goats on private lands within the Black Butte bighorn sheep population range (Rudolph et al. 2003). The population never recovered because of chronically low recruitment due to pneumonia-induced mortality in lambs (Plowright et al. 2013). By 2013, only 36 bighorn sheep were observed in surveys, and the population was estimated at 45 (Cassirer et al. 2013, Washington Department of Fish and Wildlife, unpublished data). Three

spatially distinct female groups occur in the population: Heller Bar, Shumaker, and Joseph Canyon. The groups are connected by movements of males, but we observed no female interactions across groups during this study. The Heller Bar female group was most accessible from the road and was the subject of this investigation.

METHODS

Observations

We monitored the Heller Bar female group during 2013 and 2014 as part of a study of contact patterns and lamb survival. We could individually identify 4 (31%) and 11 (85%) of the 13 adult females in 2013 and 2014, respectively, by numbered ear tags and color-coded and numbered very high frequency (VHF) radio-collars. One unmarked female was missing a horn, so all 13 sheep were individually identifiable in 2014. We located marked animals from the ground by radio-telemetry and then observed them through binoculars and a spotting scope. We conducted frequent and intensive observations between 1 May and 16 July to document productivity and neonatal survival (2013 median observation interval = 4 days, median duration of each observation = 3 hr; 2014 median observation interval = 1.5 days, median duration = 2 hr). Frequency of observation from 17 July through 26 August was every 10 days in 2013 and every 5 days in 2014, and from 26 August through the first week in October we observed the sheep once a month in 2013 and every 10 days in 2014. Median duration of observations from 17 July through October was 1 hour in both years. At each observation we recorded female and lamb health and behavior. Animals observed with nasal discharge, droopy ears, head shaking, or lethargy received a clinical score of 1, and animals observed coughing received a clinical score of 2. Sheep with no evidence of disease received a clinical score of 0.

Radio-collars on adults were equipped with a switch that triggered a fast pulse mortality signal if no movement was detected for 4 hours. We conducted site investigations, and where possible, retrieved carcasses whole when mortalities were detected. Where this was not possible, we conducted field necropsies and collected the head, the respiratory tract, and grossly abnormal tissues when available. We detected lamb mortalities through observation and retrieved whole dead lambs when autolysis was not too advanced for diagnostic testing (Cassirer and Sinclair 2007). We assigned lamb mortality dates as the midpoint between the last live observation and either the date when the carcass was found, or the date when the dam was first observed without a lamb if no carcass was located. We assumed a female had lost her lamb when it was found dead or when the number of lambs declined and she was never again observed with a lamb that year. All cadavers and tissues were submitted to the Washington Animal Disease and Diagnostic Laboratory (WADDL; Pullman, WA, USA) for analysis.

Health Sampling

We captured and sampled females in February ($n = 11$ of 13) and July ($n = 2$ of 11) 2014. In October 2014, we resampled

all 8 remaining sheep when we transferred them to captivity. We conducted captures via helicopter netgun and by darting from the ground with chemical immobilizing agents. All capture and handling followed animal care protocols approved by the Washington Department of Fish and Wildlife. Sampling entailed collecting throat swabs and placing them in buffered glycerol or Port-a-cul transport media (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA, #220144, #221606) for aerobic culture to detect Pasteurellaceae, swabbing nasal passages and placing swabs in mycoplasma broth (#102, Hardy Diagnostics, Santa Maria, CA, USA) for culture enrichment and polymerase chain reaction (PCR) detection of *M. ovipneumoniae* (Ziegler et al. 2014), and collection of serum for detection of antibodies to *M. ovipneumoniae* (competitive enzyme-linked immunosorbent assay [ELISA]), bovine respiratory syncytial virus (virus neutralization [VN]), bovine virus diarrhea (VN), infectious bovine rhinotracheitis (VN), and bovine parainfluenza-3 (PI-3, VN). Diagnostic testing on the above samples was conducted by WADDL.

We also collected upper respiratory washes by flushing nasal passages with 50 ml of phosphate-buffered saline and swabbed the oropharynx. We kept samples cool and processed them within 48 hours of collection, or stored them at -20°C . We extracted DNA (DNeasy, Qiagen, Redwood City, CA, USA) from swabs, from 10-ml aliquots of nasal wash, and from lung tissues of animals that died during the study as well as 2 pneumonic lambs that died in adjacent populations in 2013 to test for presence of pneumonia agents. We used a multiplex PCR to detect Pasteurellaceae including *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mannheimia* spp. (Besser et al. 2012b) and performed PCR for *lktA*, the gene encoding leukotoxin A, the major virulence factor of *Mannheimia* spp. and *B. trehalosi* (Walsh et al. 2016). If an agent was detected by either PCR or culture on any sample, we classified the animal as positive for that agent.

Strain Typing

Health of the Hells Canyon bighorn sheep metapopulation has been intensively monitored since the 1995 pneumonia outbreak in the Black Butte population, and intermittently prior to this. Therefore, we had access to fixed, frozen, and fresh lung tissue and swab samples collected from 1995–2015 in the Heller Bar female group and from 1986–2015 in adjacent female groups and populations. We extracted DNA from a subset of these sources to detect and strain type *M. ovipneumoniae* within the study population and the metapopulation through time. Detection was based on conventional (McAuliffe et al. 2003) and realtime (Ziegler et al. 2014) PCR.

We used multi-locus sequence typing (MLST) to characterize strains using partial DNA sequences of the 16S–23S intergenic spacer region (IGS), the small ribosomal subunit (16S), and the genes encoding RNA polymerase B (*rpoB*) and gyrase B (*gyrB*). We amplified these targets with PCR using a suite of existing and newly developed primers (Table 1). Because of the high degree of DNA sequence

Table 1. Oligonucleotide primers for polymerase chain reactions (PCR) used to amplify *Mycoplasma ovipneumoniae* multi-locus sequence typing (MLST) targets. Nesting refers to external and internal primer sets used for nested PCR reactions for amplification of the MLST loci, IGS, *rpoB*, and *gyrB*, when amplification from the default (internal) primers produced insufficient DNA template for sequencing.

Target ^a	Nesting	Oligonucleotide primer sequence	Reference
LM-F		TGAACGGAATATGTTAGCTT	McAuliffe et al. (2003)
LM-R		GACTTCATCCTGCACTCTGT	McAuliffe et al. (2003)
Ex-IGS-F	External	GTTAACCTCGGAGACCATTG	This paper
Ex-IGS-R	External	GTTTGCTAGGTTGGGTTTCC	This paper
IGS-F	Internal	GGAACACCTCCTTTCTACGG	Besser et al. (2012b)
IGS-R	Internal	CCAAGGCATCCACCAAATAC	Besser et al. (2012b)
Ex- <i>rpoB</i> -F	External	AGTTATCACAATTTATGGATCAAA	This paper
Ex- <i>rpoB</i> -R	External	GCTCAAAGTTCCATTTCCNCCGAA	This paper
<i>rpoB</i> -F	Internal	TCGGCTTCAGCAATTCCTTTCTT	This paper
<i>rpoB</i> -R	Internal	TCGGCTGTTGGGTTGTCTTCTC	This paper
Ex- <i>gyrB</i> -F	External	AAAACGWCCAGGKATGTATATTGG	This paper
Ex- <i>gyrB</i> -R	External	GGATCCATTGTTGTTTCTCATAATTG	This paper
<i>gyrB</i> -F	Internal	GGGTCAAACAAAAGCAAACTAAA	This paper
<i>gyrB</i> -R	Internal	ACGGAATAAAAATGTCAAAAGTAA	This paper

^a LM = small ribosomal subunit (16S) gene, IGS = 16S–23S intergenic spacer, *rpoB* = β subunit of RNA polymerase gene, *gyrB* = gyrase B gene, F = Forward, R = Reverse.

variation in *M. ovipneumoniae*, the biggest challenge in developing this ensemble of loci was identification of targets with sufficiently conserved primer binding site sequences to enable consistent PCR amplification for subsequent sequencing. The loci selected for this study each exhibited extensive sequence polymorphism and together provide a highly discriminatory test. We typed the concatenated sequences using multi-locus sequence analysis because of the large numbers and diversity of the alleles detected at each target locus. We aligned sequences using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>, accessed 06 Sep 2016). We report sequence divergence as the percentage non-identity.

All PCR protocols included a preliminary dissociation phase (95°C, 15 min), amplification, and a final extension phase (72°C, 7 min). We amplified the 16S target using 35 cycles of 30 seconds each of dissociation (95°C), annealing (58°C), and extension (72°C). We amplified the IGS target using 35 cycles of dissociation (95°C, 1 min), annealing (52°C, 2 min), and extension (72°C, 2 min). For nested IGS amplification, the parameters for the external primers were identical except that the annealing temperature was 54°C. For *rpoB* and *gyrB*, we used inner and nested reactions with identical amplification conditions: dissociation (95°C, 1 min), annealing (50°C, 1 min), and extension (72°C, 2 min). We treated the extracts with alkaline phosphatase and exonuclease I (FastAP and ExoI, ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. We submitted extracts to Amplicon Express (Pullman, WA, USA) to determine forward and reverse DNA sequences using the same primers used for PCR amplification. Representative sequences of all 4 target loci for the gentotypes herein are available in Genbank: strain 393 (KU986495, KU986500, KU986503, KU986506, representing IGS, 16S, *rpoB*, and *gyrB*), strain 404 (KU986496, KU986501, KU986504, KU986507), strain 415 (KU986494, KU986499, KU986502, KU986505), strain 402 (KU986493, KU986498, representing IGS and 16S), and strain 419 (KU986492, KU986497).

Statistical Analysis

We used a Kaplan–Meier staggered entry estimator and a Cox proportional hazard model to analyze survival of females and lambs. We fit trend lines to 7-day moving averages of clinical scores using a lowess smoothing factor derived from locally weighted moving mean scores spanning 25% of the full dataset (2–3 weeks). We used a Fisher's exact test to analyze prevalence of pneumonia agents before, during, and after the outbreak and to compare agents present in adult and lamb mortalities. We used a Kruskal–Wallis rank-sum test to determine whether neutralizing titers to PI-3 differed before, during, and after the outbreak. We used a 1-way analysis of variance (ANOVA) and Tukey contrasts to test for differences in serologic antibody titers to *M. ovipneumoniae* over the course of the outbreak and a 2-sided *t*-test to compare pre-outbreak antibody titers to *M. ovipneumoniae* in animals that died and those that survived. We used a Wilcoxon rank sum test with continuity correction to analyze duration of clinical signs. We conducted analyses with package survival (Therneau 2015), and stats and base packages in R (R Core Team 2015).

RESULTS

Survival and Clinical Signs of Disease

In 2013, we observed all marked females with lambs, and median parturition date was 18 May (range = 5–20 May). In 2014, we observed 10 of 13 females with live lambs and median parturition date was 8 May (range = 27 Apr–17 May). One marked female and her lamb died, presumably of dystocia, on 16 May 2014 (e5 and L5, Fig. 1a), 1 marked female was observed with a dead 2-day old lamb (e10, Fig. 1a), and 1 unmarked female appeared to be pregnant but was never observed with a lamb (e22, Fig. 1a).

We observed clinical signs of pneumonia in lambs starting 1 June in 2013 and 26 May in 2014. Symptoms continued until the day the last lamb died, which was 30 June in 2013, and 2 July in 2014 (Fig. 1). Median lamb mortality date was 28 June in 2013 and 24 June in 2014, at a median age

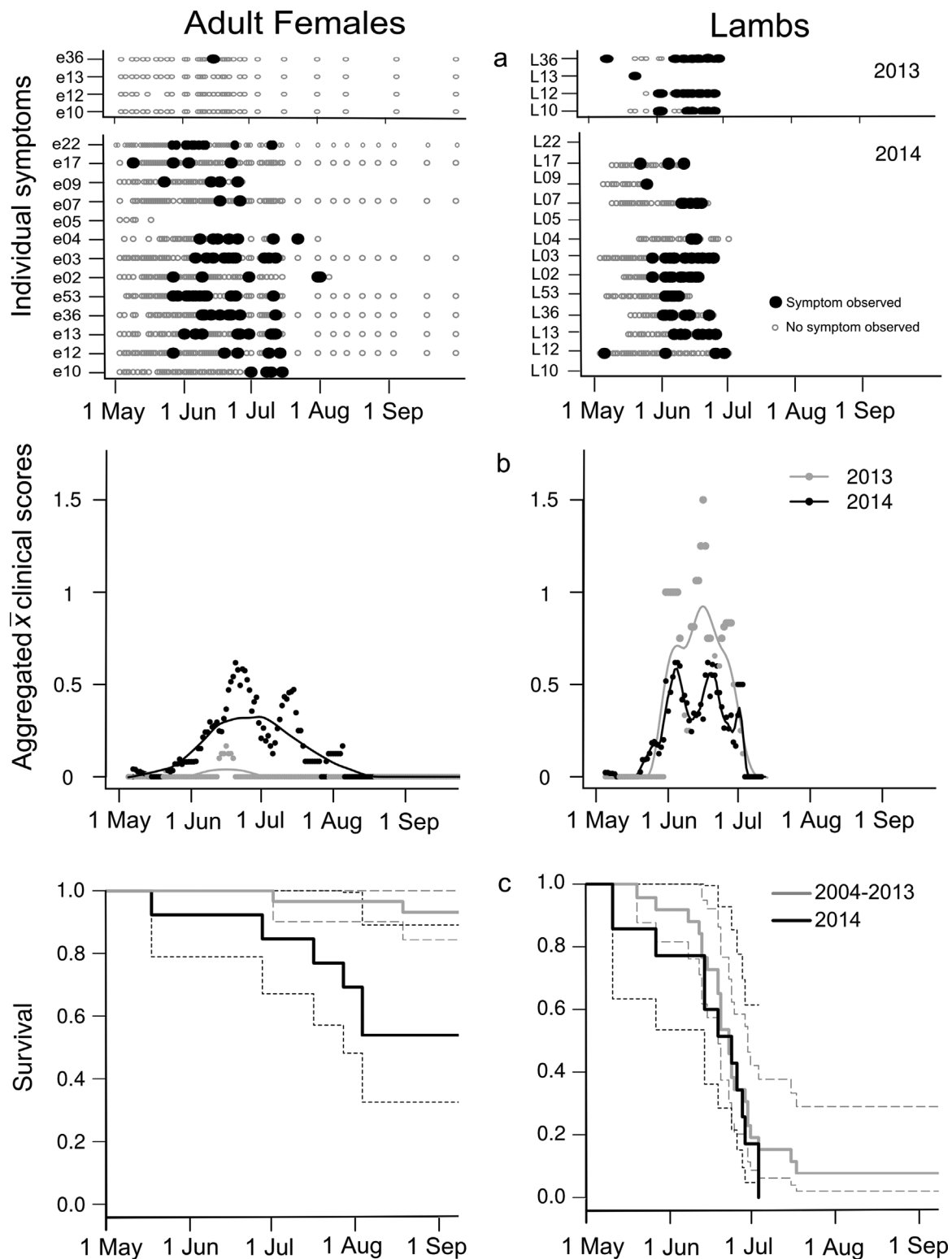


Figure 1. Clinical signs of pneumonia and survival of bighorn sheep in the Black Butte, Washington population during summers 2013 ($n = 4$ adult F and 4 lambs) and 2014 ($n = 13$ adult F and 10 lambs). (a) Time series of field observations of pneumonia symptoms in adult females (e) and their lambs (L). Observations ending prior to September indicate individual died. (b) Smoothed average daily clinical scores for adult females and lambs. (c) Adult female and lamb survival between May and October 2014 and survival between May and October during the 10 previous years, 2004–2013 (Kaplan–Meier curves and 90% CIs).

of 40 and 44 days, respectively. All lambs died both years and there were no differences in lamb survival curves between years ($\log \text{rank}_1 = 0.01$, $P = 0.92$) or between 2014 and the previous 10 years (Fig. 1c). We submitted 11 lambs

for necropsy (6 in 2013 and 5 in 2014). With the exception of 1 lamb that died at approximately 2 days of age in 2014 (L10, Fig. 1a), all lambs presented characteristic lesions of bighorn lamb respiratory disease, including moderate to

Table 2. Prevalence of *Mannheimia* spp. (Mh), *Bibersteinia trehalosi* (Bt), *Pasteurella multocida* (Pm), Pasteurellaceae leukotoxin encoding gene (*LktA*), and *Mycoplasma ovipneumoniae* (*Movi*) in asymptomatic bighorn sheep females (F) sampled before and after a pneumonia outbreak, symptomatic females during the outbreak, and in the lungs of pneumonic females that died in the Black Butte population and pneumonic lambs that died in Black Butte, (10) and adjacent populations (2), Washington and Oregon, USA.

Agent	F before (n = 11)	F during (symptomatic) (n = 2)	F pneumonia mortalities (n = 3)	Lamb pneumonia mortalities (n = 12)	F after (n = 8)
Mh	0.91	1.00	0.00	0.70	0.75
Bt	0.82	1.00	1.00	0.80	1.00
Pm	0.73	1.00	1.00	0.10	1.00
<i>LktA</i>	0.27	0.50	0.33	0.50	0.38
<i>Movi</i>	0.09	1.00	1.00	1.00	0.13

severe, subacute to chronic bronchopneumonia and otitis media.

Median age at first observation of clinical signs in lambs was 20 days (range: 7–35), median duration of clinical signs was 22 days (range: 1–59), and median age at death was 42 days (range: 22–66). Assuming that lambs were infected by 4 days of age and lung lesions were present by 10 days of age (Besser et al. 2008), we estimated a median latent period of 16 days (range: 3–31) between infection and first observation of disease symptoms in lambs and a median infection period of 38 days (range: 18–62) prior to death.

In adults, the only evidence of possible respiratory disease in 2013 was a single observation of coughing on 14 June, and all adults survived. In 2014, we observed clinical signs of pneumonia in adults starting in May. By June, all adults exhibited symptoms of pneumonia including severe prolonged coughing (Fig. 1a and b). Five adult females (38%) died between May and August 2014 (difference between summers, log rank₁ = 5.98, $P=0.01$) and the hazard of an adult female dying in the summer of 2014 was 6.83 times higher (SE = 0.83, $P=0.02$) than in any of the previous 10 summers (2004–2013, Fig. 1c). We did not observe pneumonia symptoms prior to the first adult death on 16 May 2014. Subsequent mortalities followed observation of clinical signs of pneumonia and occurred on 26 June, 18 July, 27 July, and 3 August (Fig. 1a). Median duration of clinical signs was 36 days (range = 18–81 days), which was longer than observed in lambs (median = 22 days, range = 1–59, $W=136.5$, $P<0.001$). Duration of clinical signs did not differ between adults that died and those that survived (Fig. 1a, Wilcoxon rank sum $W=20$, $P=1.0$). We submitted samples to WADDL from all (4 of 5) mortalities where sufficient tissues were available for diagnosis. No gross or histological evidence of respiratory disease was found in the female that died on 16 May, although evaluation of tissues at WADDL was limited because of autolysis. The 3 adult females submitted for necropsy between late June and early August were diagnosed with chronic, moderate to severe bronchopneumonia. The survivors appeared to make a full recovery with no evidence of ongoing disease.

Microbiology and Immune Responses

M. ovipneumoniae was the only pneumonia agent detected more frequently in the lungs of adults that died of pneumonia than in the upper respiratory tract of healthy

adults before and after the outbreak ($\chi^2_1 = 26.66$, $P<0.001$; Table 2). Prevalence of other suspected pneumonia agents in the upper respiratory tract of symptomatic adults sampled during the outbreak was similar to that detected in the lungs of adults that died of pneumonia except that *Mannheimia* spp. were detected in the upper respiratory tract of live adults but not in the lungs of adults that died. We found no differences in prevalence of pneumonia agents present in upper respiratory tract samples collected before and after the outbreak ($\chi^2_1 < 2.6$, $P>0.2$) or in the lungs of lambs that died in 2013 and 2014 ($\chi^2_1 < 0.39$, $P>0.5$). Detection of *Mannheimia* spp. was more common in lamb mortalities than in adult mortalities ($\chi^2_1 = 4.55$, $P=0.07$), and *P. multocida* was more frequently detected in the lungs of dead adults than lambs ($\chi^2_1 = 8.78$, $P=0.01$; Table 2).

We detected serologic evidence of exposure to PI-3 (VN titer ≥ 4) and *M. ovipneumoniae* (ELISA inhibition $> 50\%$) in adults before, during, and after the 2014 pneumonia outbreak. We did not detect evidence of exposure to other respiratory viruses during the study. Average *M. ovipneumoniae* ELISA percent inhibition (%I) values prior to the outbreak (69%) increased significantly to 89%I and 82%I during and after the disease event, respectively ($F_{2,18} = 4.811$, $P=0.02$; Fig. 2a). Individuals that died during the outbreak had higher *M. ovipneumoniae* ELISA % I values prior to the outbreak ($\bar{x}=79\%$) than those that survived ($\bar{x}=59\%$, $t_{7.5} = 3.02$, $P=0.02$; Fig. 2b). Median PI-3 titers of 256 (log₂ 7.7) did not change significantly over the course of the outbreak ($\chi^2_2 = 4.55$, $P=0.23$).

Strain Typing

We genotyped *M. ovipneumoniae* from all Heller Bar bighorn sheep where it was detected in 2013 ($n=7$) and 2014 ($n=11$). We found a single strain in 2013 based on identical DNA sequences of each of the 4 MLST loci, and that strain was also detected in the first lamb to die of pneumonia in 2014. All subsequent detections of *M. ovipneumoniae* differed from the 2013 strain at 3 MLST loci. The IGS sequences differed by 32 single nucleotide polymorphisms (SNP) and 3 base insertions or deletions (indels, 8.7% divergent), *rpoB* by 16 SNP (2.8% divergent) and *gyrB* by 16 SNP (4% divergent). The 16S sequences did not differ between strains.

We report strain differences by IGS sequences because we were unable to amplify *rpoB* and *gyrB* from DNA extracted

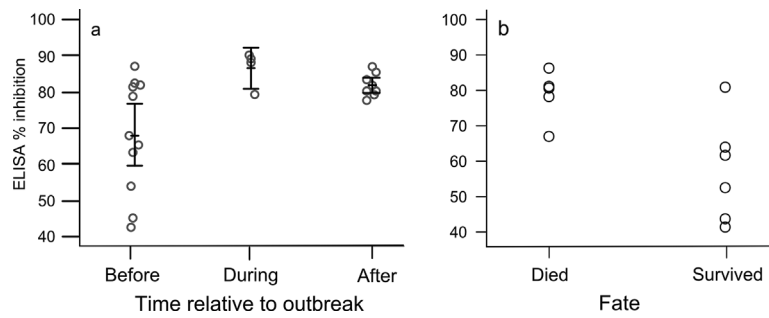


Figure 2. Serologic titers (competitive enzyme-linked immunosorbent assay [ELISA]) to *Mycoplasma ovipneumoniae* in bighorn sheep in Black Butte, Washington, February–October 2014. (a) Mean and 90% confidence intervals before, during, and after a pneumonia outbreak. (b) Antibody titer levels before the outbreak in adults that survived ($\bar{x}$ = 59%) and those that died ($\bar{x}$ = 79%).

from formalin-fixed, paraffin-embedded lung tissues, the only specimens available prior to 2006. Because each of these strains differed by indels in IGS, the strains are conveniently designated by their differing IGS lengths. The 2013 Black Butte strain, IGS 404, has been detected in this population since the 1995 pneumonia outbreak (Fig. 3b). The 2014 strain, IGS 393, had never previously been detected in this population or any other bighorn sheep population in Hells Canyon or elsewhere in the western

United States (among >700 other isolates that have been IGS typed).

In October 2014, we removed survivors from the Heller Bar female group in an attempt to prevent further spread of this strain. Nonetheless, 1 month after the removal, we detected the IGS 393 strain type in an adult male removed from the town of Asotin, Washington and we detected it again 4 months later in a 9-month-old lamb in the Shumaker female group, located between the Joseph and Heller Bar

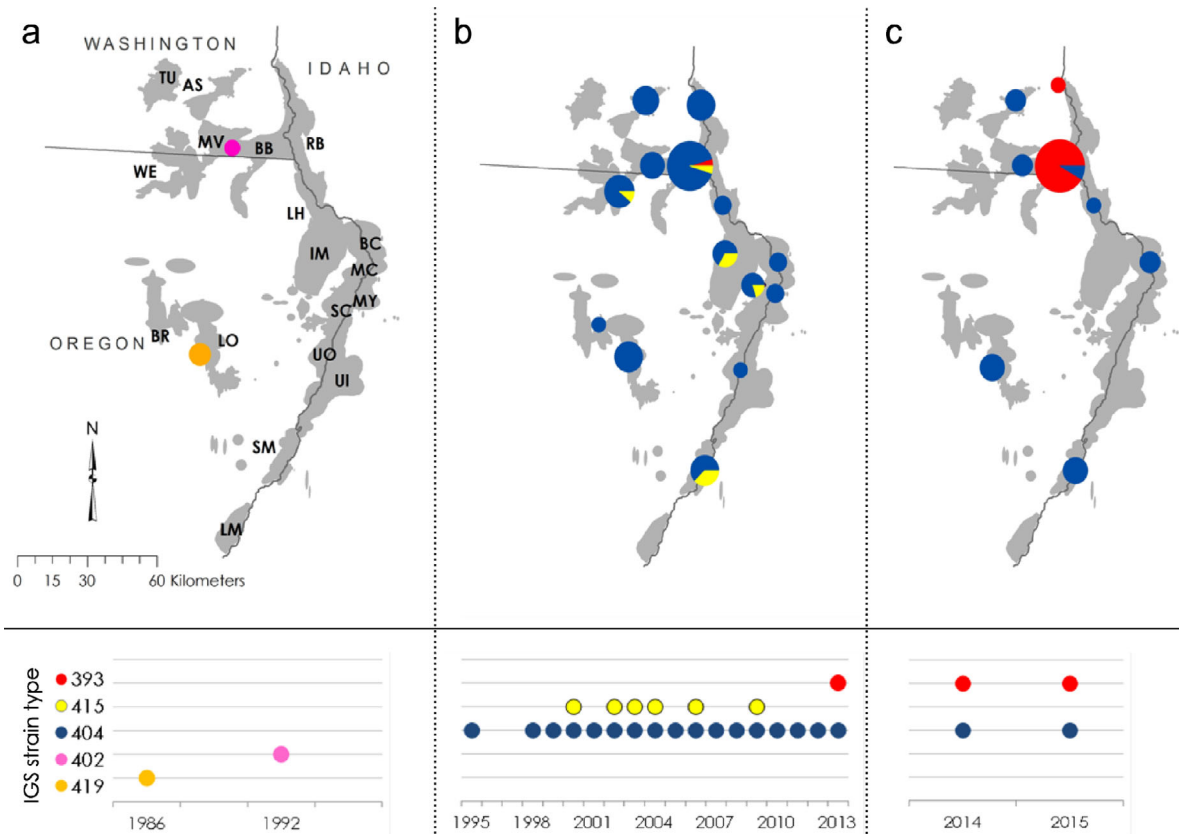


Figure 3. Spatiotemporal distribution of the 16S-23S intergenic spacer (IGS) genotypes of *Mycoplasma ovipneumoniae* in the Hells Canyon bighorn sheep metapopulation in Washington, Oregon, and Idaho, USA in (a) 1986 and 1992 (b) 1995–2013; and (c) 2014–2015. Each colored marker represents one strain type, pie charts display strain types within populations and are scaled by sample size. Pie charts containing >1 color indicate that 2 strain types were present in a population during that time interval but not necessarily detected in the same year. Gray shaded polygons denote bighorn sheep populations: AS = Asotin, BB = Black Butte, BC = Big Canyon, BR = Bear Creek, IM = Imnaha, LH = Lower Hells Canyon, LM = Lookout Mountain, MU = Muir, MV = Mountain View, MY = Myers Creek, RB = Redbird, SM = Sheep Mountain, TU = Tucannon, UO = Upper Hells Canyon, Oregon, UI = Upper Hells Canyon Idaho.

groups in the Black Butte population. Retrospective analysis revealed that this strain was present in the lungs of a male that died of pneumonia in the Joseph Creek group in December 2013, representing the index case of disease associated with the IGS 393 strain type. The IGS 393 strain has not been detected in any surrounding populations, which remain carriers of the original IGS 404 strain (Fig. 3c).

Two other strains were detected in specimens obtained during or after disease outbreaks in other Hells Canyon populations (Coggins 1988, Foreyt et al. 1990), and have not been detected since. These samples were from the Lostine population in 1986–1987 (IGS 419) and the Mountain View population in 1992 (IGS 402; Fig. 3a). A third strain (IGS 415) associated with a pneumonia outbreak in the Sheep Mountain population in 2000 was apparently replaced by the IGS 404 strain by 2006 based on samples typed from 2006 and 2015 in that population. The IGS 415 strain was subsequently infrequently detected in 4 other populations between 2003 and 2009 and has not been found since then (Fig. 3b).

Sequence divergence among the 3 recently detected strains where all 4 genes could be analyzed (i.e., IGS 393, 404, and 415) was between 9% and 10%. These strains were well dispersed across 20 genotypes of *M. ovipneumoniae* collected from 9 domestic sheep flocks in the western United States and Australia and 10 domestic goat flocks in the western United States and China. The IGS 404 and 415 types, first identified in the Black Butte and Sheep Mountain populations, respectively, were more closely related to the domestic sheep lineage, whereas the IGS 393 type detected in the Black Butte population in 2013 and 2014 clustered with the domestic goat clade (Fig. 4).

DISCUSSION

This is the first study to report on an all-age pneumonia outbreak in an intensively sampled population of free-ranging bighorn sheep with health, survival, and observational data collected before, during, and after the disease event. The collection and analysis of this information, employing recently developed molecular methods for pathogen detection and genotyping, allowed us to attribute severe disease in a bighorn sheep population with long-standing *M. ovipneumoniae* carriage to introduction of a novel strain of *M. ovipneumoniae*. This conclusion is supported by the detection of the never before recorded IGS 393 strain in the pneumonic lungs of adults that died, and in the upper respiratory tract of adults with clinical signs during the outbreak. We also observed a significant increase in antibody titers to *M. ovipneumoniae* during the outbreak denoting an active immune challenge and we documented that previous exposure and ongoing carriage of the *M. ovipneumoniae* IGS 404 strain was not protective against disease. To the contrary, lower survival of adults with higher serologic titers prior to the disease outbreak could reflect a harmful autoimmune reaction associated with antibody response to *M. ovipneumoniae* in bighorn sheep as has been suggested for domestic sheep (Niang et al. 1998a). Strain-specific immunity, as measured by serologic antibody

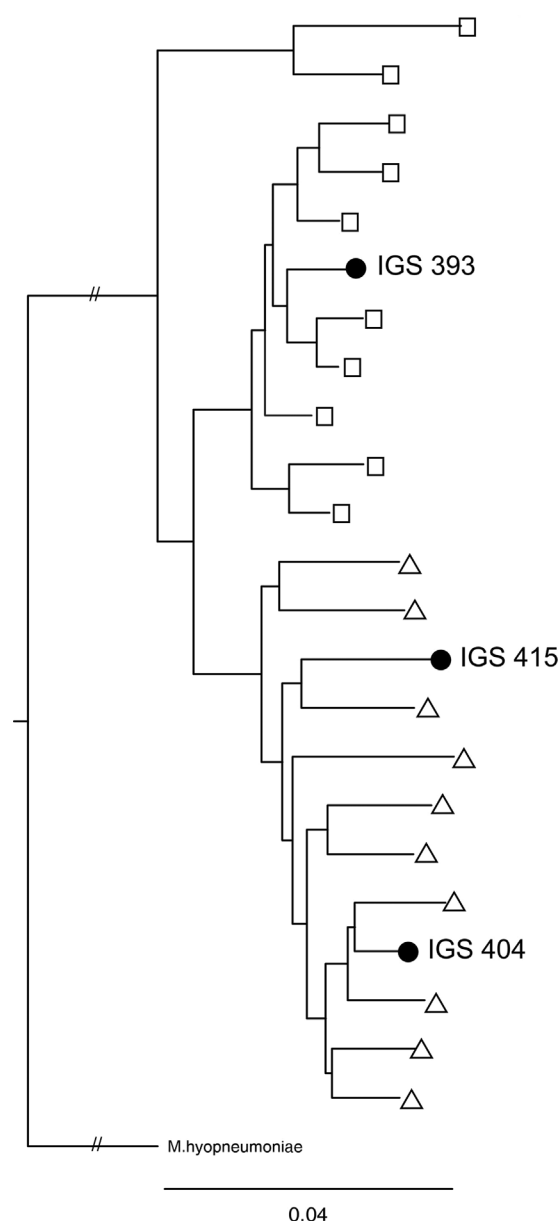


Figure 4. Phylogeny of 3 bighorn sheep strains of *Mycoplasma ovipneumoniae*, Hells Canyon, Washington, Oregon, and Idaho, USA, and randomly selected strains from domestic sheep (triangles) and domestic goats (squares), 1975–2015. Domestic sheep and goats appear to host divergent lineages of *M. ovipneumoniae* and the samples from bighorn sheep clustered with either the domestic sheep or domestic goat lineages. Neighbor-joining tree of concatenated partial 16S, 16S-23S intergenic spacer region (IGS), *rpoB*, and *gyrB* DNA sequences is rooted with homologous sequences from *M. hyopneumoniae*. Branch lengths are scaled by proportion non-identity (scale bar). Domestic sheep samples are from Washington, Oregon, Idaho, Nevada, Colorado, California, and an ATCC type strain from Australia. Domestic goat samples are from Washington, Idaho, California, and China (Yang et al. 2011, 2014).

inhibition of *M. ovipneumoniae*, was similarly reported by Alley et al. (1999) for domestic sheep.

Adult mortality associated with this strain introduction was within the range previously observed during pneumonia outbreaks in naïve animals in this metapopulation (28–42%; Cassirer et al. 2013). Lamb mortality followed an identical time course regardless of strain, consistent with a lack of

protective immunity in neonates. The timing of the onset of clinical signs following infection in lambs (latent period) was similar to that reported in experimental challenge of adults (Besser et al. 2014). However, disease progression in lambs was more rapid and severe than observed in free-ranging adults in this study or in experimental exposure of naïve adults in captivity (Besser et al. 2014).

Although pneumonia in bighorn sheep is a polymicrobial disease, pathogens other than *M. ovipneumoniae*, including *lktA* positive *Pasteurellaceae* and respiratory viruses, were either not detected or showed no association with disease. Whereas *M. ovipneumoniae* was present in all pneumonic adults and lambs, prevalence of *Pasteurellaceae* varied between age classes. This could be due to conditions associated with growth of an opportunistic pathogen or to other factors. Sample sizes were too small to draw broader inference. Anaerobic bacteria, not tested in this study, are a large component of the microbiome in pneumonic bighorn sheep lung tissue and may also play a larger role as secondary pathogens than previously suspected (Besser et al. 2008).

The novel strain of *M. ovipneumoniae* detected in this study differed from the resident strain by 52 independent genetic mutations on 4 loci. This unique strain was not a variant of a resident strain and had never before been detected in over 700 samples strain-typed from Hells Canyon and other bighorn sheep populations. Therefore, the most likely source of this Caprinae-specific pathogen was a domestic sheep or goat. Phylogenetic analysis indicated that this strain was most likely of domestic goat origin.

This strain introduction likely occurred from a domestic goat on or from private lands within bighorn sheep range despite substantial efforts by wildlife managers and nongovernmental organizations to prevent contact. Management strategies included distributing educational material to flock owners and the general public, purchasing and removing a domestic sheep flock, and removing individual bighorn or domestic sheep and goats when they were at risk of contact. Our retrospective analysis showed that unique strains of *M. ovipneumoniae* were associated with 4 other epidemiologically unrelated all-age pneumonia outbreaks in Hells Canyon, as would be expected from similar spillover events. Two of these historical strains (IGS 402 and 419) apparently remained localized, one spread and subsequently disappeared (IGS 415), whereas the fourth (IGS 404) has persisted and proliferated over a span of ≥ 20 years. It is not clear why some strains of *M. ovipneumoniae* persist and others apparently do not. The IGS 404 strain may have driven fade-out of other extant strains when it spread, as introduction of the IGS 393 strain did in this study. Strain replacement might occur when the carrier host immune response is cross-reactive but protection is strain-specific. Under these conditions a new strain could have a competitive advantage and exclude the original strain.

Although evidence suggests that this epizootic was caused by introduction of a novel *M. ovipneumoniae* strain, it is also possible that pneumonia outbreaks could be precipitated in carrier bighorn sheep populations if appropriate mutations occur in strains to which resistance has previously been

acquired. Mutations in key virulence genes or in genes coding for the *M. ovipneumoniae* capsule, which likely plays a role in adherence to host cells and in evading antibody recognition (Niang et al. 1998b, Razin et al. 1998), could cause disease and are unlikely to be detected by our strain-typing method. Another plausible mechanism of pneumonia resurgence would be reintroduction of the same strain of *M. ovipneumoniae* into a population following either pathogen fade-out and waning immunity (Sydenstricker et al. 2005) or recruitment of unexposed susceptible individuals. Finally, other factors may play a role in triggering outbreaks such as pathogen dose, host contact patterns, immunocompetence, and invasion of secondary pathogens. Identifying the conditions most frequently associated with pneumonia outbreaks in previously exposed populations and a wider investigation of the genetic diversity and host-specificity of *M. ovipneumoniae* strains would provide valuable insights into the adaptive immune response in bighorn sheep and the ecology of this disease.

MANAGEMENT IMPLICATIONS

Lack of cross-strain immunity to *M. ovipneumoniae* could be one explanation for the regular occurrence of pneumonia epizootics in bighorn sheep populations over a century after initial contact with domestic sheep. Single strain infection in bighorn sheep populations contrasts with *M. ovipneumoniae* carriage in domestic sheep where numerous strains typically coexist within a flock (Alley et al. 1999, Harvey et al. 2007). In the absence of cross-strain immunity, these flocks may serve as a constant source of novel strains capable of causing disease in bighorn sheep. Although vaccination could potentially reduce pathogen burden or prevalence within bighorn sheep populations, it is not clear that a vaccine would protect bighorn sheep from severe disease if exposed to new strains. Our results instead support preventing spillover as a primary strategy for managing disease in bighorn sheep. This could be accomplished by maintaining separation between bighorn sheep and domestic sheep and goats, by clearing *M. ovipneumoniae* infection from domestic hosts, and by exercising caution to avoid mixing *M. ovipneumoniae* strains among bighorn sheep populations during translocations. The management strategies implemented near the bighorn sheep in this study were apparently unsuccessful in preventing transmission, underscoring the difficulty of maintaining separation. New approaches, more active cooperation by the public, and greater vigilance on the part of resource managers may be key to preventing pneumonia outbreaks in bighorn sheep.

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RISK FACTORS AND PRODUCTIVITY LOSSES ASSOCIATED WITH *Mycoplasma ovipneumoniae* INFECTION IN UNITED STATES DOMESTIC SHEEP OPERATIONS

A PREPRINT

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ABSTRACT

Association of *Mycoplasma ovipneumoniae* with pneumonia in domestic small ruminants has been described in Europe, Asia, and New Zealand but has received less attention in the United States. In 2011, the US Department of Agriculture’s National Animal Health Monitoring Survey detected *M. ovipneumoniae* shedding in 88% of 453 domestic sheep operations tested in 22 states that accounted for 85.5% of US ewe inventory in 2001. We evaluated factors associated with *M. ovipneumoniae* infection presence and prevalence, and we compared health, lamb production, and ewe losses in infected and uninfected operations. *M. ovipneumoniae* detection was more common in larger operations than in smaller operations. Both likelihood of detection (at the operation level) and within-operation prevalence were higher in operations with more open management practices than in operations with more closed management practices. *M. ovipneumoniae*-positive operations showed significantly lower lambing rates and lower rates of lamb survival to weaning after accounting for differences in operation size and management practice. While its effect on any single rate was not particularly large, in aggregate we estimated that *M. ovipneumoniae* presence was associated with an approximately 4.3% reduction in annual lamb production.

Keywords sheep, respiratory disease · *Ovis aries* · *Mycoplasma ovipneumoniae* · productivity · prevalence

1 Introduction

Mycoplasma ovipneumoniae is a recognized pathogen of Caprinae [1, 2, 3], and has been associated with transmissible respiratory disease in domestic sheep in experimental settings [3]. Like other respiratory mycoplasmas, *M. ovipneumoniae* initially colonizes the respiratory tract, where it impedes movement of the ciliary escalator [4, 5]. This allows a variety of normally commensal upper respiratory, rumen, and inhaled flora to invade the lungs, where they can cause chronic, polymicrobial pneumonia [6, 4, 7].

Veterinary texts describe *M. ovipneumoniae*’s impact on animal health and operation productivity with characterizations ranging from “mild” (in Smith’s Large Animal Internal Medicine[8]) to “proliferative” (in Radostits’s Veterinary Medicine [9]). The diversity of descriptors leaves ambiguity about how veterinarians and operators should view *M. ovipneumoniae* infection in terms of production loss. Respiratory disease is a serious problem for domestic sheep production in the United States, and is the fifth-highest source of lamb loss, following weather, lambing problems, predation, or unknown causes, and on a par with gastrointestinal problems [10, 11]. Additionally, there are only limited and local data on *M. ovipneumoniae* presence in United States domestic sheep operations [6, 12, 13, ?]. A better understanding of the risk factors associated with *M. ovipneumoniae* infections, and the burden these infections place on domestic sheep production would help producers determine how to prioritize its management.

Here, we investigate whether *M. ovipneumoniae* is associated with reduced productivity in United States domestic sheep operations. We postulate that *M. ovipneumoniae* may be an under-recognized pathogen of domestic sheep in the United States for two reasons. First, disease burdens associated with other *Mycoplasma* infections of livestock are well-documented (e.g., [14]). This is true, for example, of *Mycoplasma mycoides*, the causal agent of contagious bovine pleuropneumonia, and *Mycoplasma capricolum*, the causal agent of contagious caprine pleuropneumonia, as well as the disease burden associated with *M. ovipneumoniae*’s close relative *Mycoplasma hyopneumoniae*, a pathogen of pigs (e.g., [15, 16]). Second, *M. ovipneumoniae* is thought to cause chronic disease and generate important mortality burdens in domestic sheep in other parts of the world [3, 2]; as well as in other caprine hosts, including domestic goats [17, 18]. However, despite burdens associated with *M. ovipneumoniae*’s close relatives in domestic animals, and with *M. ovipneumoniae* itself in other caprinae hosts, little work has explored the burden of *M. ovipneumoniae* on domestic sheep production within the United States.

We describe the distribution of *M. ovipneumoniae* in domestic sheep operations throughout the United States using data acquired through the U. S. Department of Agriculture National Animal Health Monitoring System. Our inquiry is built around two questions. First, can we identify specific management practices associated with *M. ovipneumoniae* infection that would allow us to predict — and potentially reduce — infection status and prevalence? Second, what are the consequences of *M. ovipneumoniae* infection on specific production and aggregate productivity for United States domestic sheep operations?

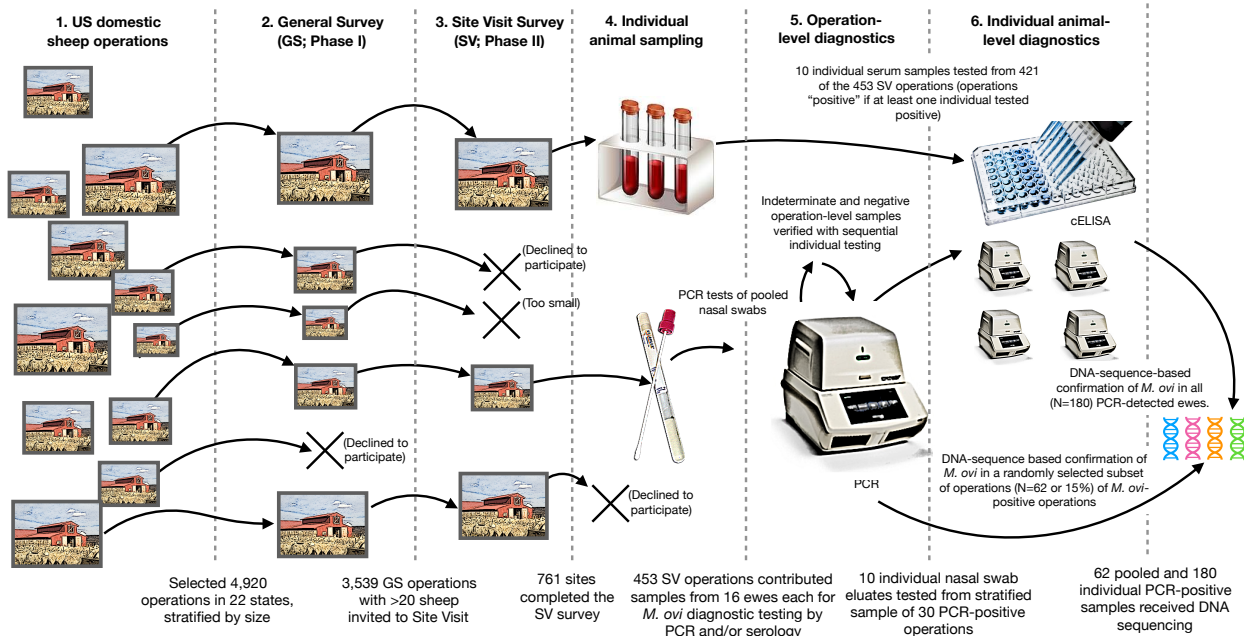


Figure 1: **Sampling and data development.** Operations were identified prior to Phase I of the NAHMS Sheep-2011 survey, and a size-stratified sample of 4,920 operations were invited to participate in the General Survey Phase I. A subset of General Survey Phase I operations elected to participate in the Site Visit (Phase II) portion. Of that subset, a smaller group (761) completed the survey. Of these, 453 participated in the *Mycoplasma ovipneumoniae* portion of the study. Sixteen animals at each of those 453 operations contributed nasal swabs and venous blood samples. Those samples were developed individually, and then pooled and tested by PCR and cELISA. When an operation's pooled PCR result was indeterminate or negative, individual samples from that operation were tested sequentially until an animal tested PCR-positive, or all tests were queried. Ten animals each from a structured subsample of 30 operations PCR-positive on the pooled test were then tested at the individual level to establish operation-level prevalence estimates. A random subset of 62 pooled operation samples, and all 180 individual animal samples that tested PCR-positive in the prevalence estimation were then sequence-confirmed as being *M. ovipneumoniae*-positive.

2 Materials and methods

2.1 Data collection and laboratory analyses

2.1.1 USDA National Animal Health Monitoring Survey (NAHMS) Data

Data were collected through the USDA-APHIS-VS National Animal Health Monitoring System (NAHMS) on domestic sheep production conducted in 2011 (Figure 1). Like most NAHMS studies [19, 20], the survey consisted of two stages. First, premises were selected from a sampling frame of all domestic sheep operations (those with one or more sheep on hand on January 1, 2010; [21]) in 22 states accounting for 85.5% of United States domestic sheep production [21]. A sample of 4,920 operations, stratified by state and operation size, was drawn from this frame. Participants completed a General Survey ("GS"; Phase I) questionnaire between January 1st and February 11th, 2011 (USDA 2012), which covered a range of issues relevant to domestic sheep production. As in other NAHMS surveys [19, 20], the 3,539 Phase I operations with 20 or more ewes (71.9% of all GS operations) were then invited to participate in a second site visit ("SV"; Phase II) phase of the study, which included sample collection. One thousand, two hundred and forty-one (35.1%) of the GS operations with 20 or more animals agreed to participate in the site visit portion of the survey ([10] pg. 145), of which 761 (61.3%) actually contributed complete information ([10] pg. 150; Figure 1). Of those, 453 operations (59.5%) also contributed the biological samples forming the basis for the analysis presented here.

2.1.2 Collecting samples from domestic sheep operations

At each participating operation, the Veterinary Medicine Officer conducting the Phase II site visit obtained nasal swab samples from 16 ewes (Figure 1). This sample size was chosen by NAHMS to balance pre-specified precision estimates

for detecting *M. ovipneumoniae* presence (not prevalence) given estimated flock and animal-level prevalence against necessary budget constraints. A single swab (BBL CultureSwab EZ 220144) was used to swipe both nares from each sampled animal, and was then placed in mycoplasma transport media. All 16 nasal swabs from each operation were shipped to the Washington Animal Disease Diagnostic Laboratory (WADDL). Blood samples (10 ml) were obtained by jugular venipuncture from the same 16 sampled ewes, collected into red top (clot) tubes, and shipped to the National Veterinary Services Laboratory, where serum was extracted and aliquoted. One set of serum aliquots was then shipped to WADDL for testing.

2.1.3 Detecting *Mycoplasma ovipneumoniae* by PCR

At WADDL, nasal swabs were placed in mycoplasma broth media (R102, Mycoplasma Broth, Hardy Labs, Santa Maria CA) and incubated at 35C for 72-96 hrs. Following incubation, aliquots of post-enrichment broths from the 16 ewes were pooled into a single operation-level sample. DNA was extracted from the pooled sample (MagMAX, ThermoFisher Scientific, Waltham MA) and tested using PCR for *M. ovipneumoniae* [22]. Operations with pooled samples producing cycle threshold (CT) values of 34 or less were classified as “infected”. If pooled samples produced CT values >34, individual nasal swab enrichment tube samples were tested. Operations in which all pooled and individual nasal swab enrichment tube samples produced CT values >34 were classified as “not infected” (Figure 1).

To determine operation-level prevalence of *M. ovipneumoniae* carriage, 30 operations were randomly selected for realtime PCR testing of mycoplasma enrichment broths from ten ewes each. After initial analysis of the specimens from this project in 2011, the realtime PCR method for detection of *M. ovipneumoniae* was modified to eliminate false positive reactions associated with the presence of an unidentified Mycoplasma-like organism. To ensure consistency in diagnostic methods, this modified realtime PCR method was used to retest all available DNA extracts in which *M. ovipneumoniae* had originally been detected. The modified version of the WADDL realtime PCR included 1) use of a new primer (226Fnew 5'-GGGGTGCACAACTAGTTAGTTGGTAG-3') to replace the previously described forward primer [22], 2) modified master mix to include bovine serum albumin (final concentration, 400 ng/ml), and 3) modified thermocycling conditions including Stage 1: 50C hold for 2 minutes followed by 95C hold for 10 minutes; optics off; Stage 2: 40 repeat cycles of denaturation (95 C, 15 seconds, optics off) and annealing/extension (66 C, 60 seconds, optics on). The results of the modified realtime PCR were interpreted as follows: ‘detected’ if the cycle threshold score (CT) was 36 or lower, ‘indeterminate’ for CTs between 36 and 40, and ‘not detected’ for a CT of 40.

Verifying accuracy of *M. ovipneumoniae* detection

To ensure consistency of *M. ovipneumoniae* detection, the modified WADDL realtime PCR described previously was also used to re-test specimens from all available operations classified as *M. ovipneumoniae*-positive based on the WADDL 2011 test method. Of the 401 operations classified as *M. ovipneumoniae*-positive based on the 2011 test, DNA specimens were available for re-testing from 372 operations.

Both the original and the modified methods were also used for determining sequences of multiple loci for comparison with reference strain data [23]. In addition, for some samples a different partial 16S rDNA target sequence was used, amplified by the primers 226Fnew and 1025Rnew (5'-ATCTCGTTAGCCTCCGCTATATCT-3') with the thermocycling conditions Stage 1: 95C hold for 5 minutes; Stage 2: 45 cycles of denaturation (95 C, 30 seconds), annealing (66.5C, 30 seconds) and extension (72 C, 60 seconds); and State 3: 72C hold for 10 minutes. Sequence confirmation was sought from DNA extracts from the pooled operation-level enrichment broths from a randomly selected subset of 62 *M. ovipneumoniae*-infected operations. Sequences obtained were used to search GenBank using the Basic Local Alignment Search Tool (BLAST) to detect the most similar database entries. In addition, DNA sequence confirmation was applied to DNA extracts (N=180), which included all individual ewe samples from the 30 randomly selected operations in which *M. ovipneumoniae* was detected by the modified WADDL realtime PCR method. DNA sequences resulting from these efforts have been deposited in GenBank (MH042304-MH042516; MH045511-MH045514; MH087248-MH087420; MH107389-MH107763).

2.2 Data analysis

2.2.1 Response variables

The analysis was built around two separate blocks of response variables. In the first block, *M. ovipneumoniae* was treated as a response, and we analyzed *M. ovipneumoniae* presence (0 or 1, based on the pooled operation-level samples) and, given a positive operation, prevalence (proportion 10 ewe samples that tested PCR-positive, for the subset of operations receiving follow-up testing) as a function of a set of potential risk factors. In the second block, *M. ovipneumoniae* was treated as a predictor, and we explored how ewe survival and productivity was measured by number of pregnant ewes lambing at full term, number of lambs born per ewes bred, number of birthed lambs surviving to weaning, lamb weight

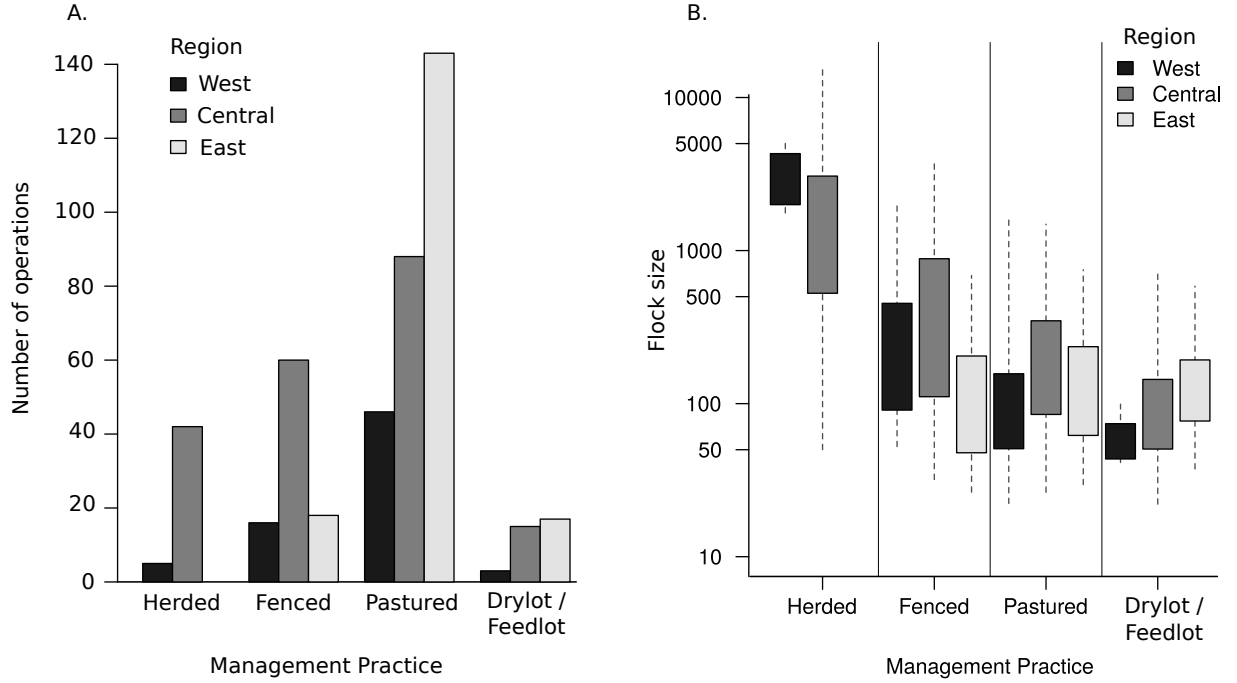


Figure 2: **Sheep management styles.** (a) Distribution of region-level flock management practices across 453 operations that contributed biological samples during the Site Visit portion of the NAHMS Sheep-2011 survey. (b) Logged herd size across four different herd management practices, and split out by region. Boxes capture the middle 50% of operations in each group; lines extend to the 2.5th and 97.5th quantile in each group.

at weaning, and number of sheep dying from respiratory disease — varied according to *M. ovipneumoniae* presence and prevalence.

2.2.2 Potential predictors of *M. ovipneumoniae* infection

We considered five risk factors that we hypothesized could influence a flock’s likelihood of being infected with *M. ovipneumoniae*, and might affect *M. ovipneumoniae* prevalence within infected flocks. The risk factors were 1) operation size; 2) primary management type; 3) an index for operation biosecurity (ranging -1 to 10); 4) an index for overall disease burden (ranging 0 to 11); and 5) an index for operation-level antibiotic use practices (ranging 0 to 5). Primary management types were “fenced” operations where animals were contained in any fenced area not specifically cultivated to raise forage or browse; “herded” operations where animals were maintained on any unfenced acreage; or “pastured” operations where animals grazed fenced areas specifically cultivated to raise forage or browse [21]. Data from 35 dry lot and feedlot operations that contributed biological samples through the Site Visit portion of the study but that did not allow grazing were excluded due to limited occurrence in our dataset. The three index variables were created by aggregating each operation’s responses across a series of General Survey and Site Visit questions. Contributing survey questions are listed in Table S1, and univariate distributions of each index are shown along the main diagonal of Figure S1.

Regions varied substantially in management type and operation size (Figure 2), which confounded management type and region. In particular, operations that pastured their animals were disproportionately located in the East region, whereas operations that used fenced or herded management practices were disproportionately located in the Central region (Figure 2). Since there is no clear mechanism for regional difference in *M. ovipneumoniae* persistence outside the host (*M. ovipneumoniae*’s environmental persistence is thought to be uniformly poor due to its lack of a cell wall, [24], and in an effort to focus our analyses and protect participant privacy, we chose to omit Region throughout the remainder of this investigation.

We first evaluated univariate relationships between each risk factor and *M. ovipneumoniae* presence across all fenced, pastured, and herded operations contributing biological samples to NAHMS Sheep 2011 Phase II using Wilcoxon rank-sum tests, and then we fit a logistic regression model in which operations were assigned a one if they were *M.*

ovipneumoniae-positive, and a zero otherwise. All covariates were included as additive effects, since a subset of models examining covariate interactions showed limited, or no improvement in model performance on the basis of AIC (all models with interactions were > 2 AIC points above model with only additive terms). Covariates were not substantially collinear with one another (absolute value of all correlations < 0.4 throughout; see Figure S1), so we included them all in subsequent models. An identical model with a binomial residual structure with $n = 10$ (number of animals individually tested by PCR in each operation) was used to characterize associations between the same set of risk factors and *M. ovipneumoniae* prevalence on the subset of 62 size-stratified operations subject to individual-level testing (see Detecting *Mycoplasma ovipneumoniae* by PCR).

2.2.3 Associations between vital rates and *M. ovipneumoniae* infection status

We examined the association of operation-level *M. ovipneumoniae* presence (zero for no *M. ovipneumoniae* detection) and prevalence in positive operations (number of individuals testing positive out of 10) with seven response variables linked to productivity and animal health. Response variables were full-term births among pregnant ewes, number of lambs born per bred ewe, lambs born that survived to weaning, average lamb weight at weaning, number of ewes culled, and number of ewes and lambs that died from respiratory disease. We built one set of models in which *M. ovipneumoniae* presence or absence was a predictor (fit to all operations with complete biological samples), and a second set in which *M. ovipneumoniae* prevalence was a predictor (fit to prevalence data from the stratified set of 62 operations where individual-level prevalences were established). All models also contained management type and operation size covariates to account for vital rate differences among production styles. We checked for correlations among transformed response variables, and they were negligible following transformation (Figure S4).

The proportion of pregnant ewes that deliver lambs at full term was treated as a binomial variable. The number of lambs born per bred ewe was treated as Poisson, with total number of lambs born. Lamb survival to weaning was treated as a binomial response variable. Lamb weight at weaning was modeled as Gaussian with an identity link; that model also incorporated average age at weaning, and an indicator variable of whether lambs were fed high-energy diets. We also examined the number of ewe, pre-weaning lamb, and weaned lamb deaths due to respiratory disease. These were treated as negative binomial variables to account for over-dispersion in the raw counts (many operations had no respiratory deaths in any age group). The negative binomial models also included total number of sheep on the operation as offset terms.

Additional descriptions of response variables, formal statements of all models, and specific model fits, are included in the Supplementary Materials.

2.2.4 Statistical analyses

All statistical analyses were conducted in R [25]. Linear and most of the generalized linear models were fit using the `bayesglm` function in the `arm` package [26] to circumvent any issues associated with complete separation – the situation in which a covariate completely splits a response, such that all positive (or negative) responses occur under a single level of the covariate. This might occur, for instance, if all operations of a particular management type tested PCR-positive for *M. ovipneumoniae*. `bayesglm` works as a simple augmentation of the usual iteratively reweighted least squares that is classically used to fit generalized linear models by first incorporating a small data augmentation to adjust for complete separation, and then running a step of iteratively weighted least squares and a step of expectation-maximization algorithm to estimate coefficient variances [27]. While the inference approach is fundamentally Bayesian in that it directly incorporates priors, it is structured to analogize cleanly with iteratively reweighted least squares; thus our inferences are presented as a hybrid of Bayesian and frequentist norms. Student's T-distributions with 2.5 degrees of freedom (or 10 degrees of freedom in the case of the intercept) for all coefficients with expectation-maximization updates were used as priors for all model coefficients.

To model over-dispersed count variables (e.g., count variables for which there are more zeroes than expected), negative binomial regression models were fit using the `bayes_glm` function in the `rstanarm` package [28]. Negative binomial models are typically chosen as alternative models for over-dispersed data because they naturally account for the extra-Poisson variability via an additional variance parameter [29, 30]. The `bayes_glm` function fits Bayesian generalized linear regression models in the Stan programming language [31], which uses adaptive Hamiltonian Monte Carlo sampling to simulate draws from the posterior distribution. These are fully Bayesian models, unlike those fit by the `bayesglm` function and thus do not generate p-values. Instead, posterior credible intervals are used to make inference about predictor variables of interest. Again, Student's T-distributions with 2.5 degrees of freedom (or 10 degrees of freedom in the case of the intercept) was used as the prior distributions for all model coefficients.

During a preliminary investigation, we relied on AIC-based model selection to identify best-performing models, and considered models with all possible combinations of covariates in the model suites. This exploration indicated that

models including additive effects of operation size and management nearly always performed best, and we used that covariate structure as the basis for all models presented here. We report McFadden's pseudo R-squared values as a coarse metric of performance on logistic regression model fits. Though widely used, pseudo R-squared values are not identical to conventional R-squared values available through ordinary least squares. Pseudo R-squared values are often lower than conventional R-squared values, and in general, values of 0.2 - 0.4 are considered "excellent fits" [32].

3 Results

3.1 Data overview and structure of sampled operations

A total of 453 operations voluntarily contributed biological samples for *M. ovipneumoniae* testing as part of the Sheep 2011 study. Ninety-two percent (418) of those operations were under fenced, pastured, or herded management practices, and of those 418, 10 contributed swabs but not serum samples. A cross-tabulation of operations where *M. ovipneumoniae* was detected via PCR, and where antibodies to *M. ovipneumoniae* were detected via cELISA, is shown in Table S2, along with a full description of cELISA methods.

Operation size and production type varied by region. Herding occurred in the Central and Western regions of United States, but not in the East. Operations using herded management systems were on average much larger (median = 1,750 animals; 95% of operations between 50 and 15,200 animals) than operations using pastured (median = 128 animals; 95% of operations between 26 and 1,444 animals) or fenced (median = 195 animals; 95% of operations between 31 and 2,867 animals) management. Although only 3.1% of operations used herded or open-range management, those operations accounted for 26.1% of ewes on operations with 20 or more animals (USDA-APHIS 2012). A comparison of the NAHMS Site Visit sites with data from all operations surveyed during the Sheep 2011 NAHMS General Survey suggested that Site Visit sites provided a reasonable cross-section of production sizes and management strategies used across all surveyed sites.

3.2 Reliability of diagnostic tests

M. ovipneumoniae was detected by the modified realtime PCR method in 369 of the 372 available specimens. Of the three discrepant samples, one produced 'indeterminate' results by the modified realtime PCR, while *M. ovipneumoniae* was not detected in the other two specimens by either the original or the modified realtime PCRs, suggesting that the DNA template had degraded during storage. Overall, the modified realtime PCR method detected *M. ovipneumoniae* in 369 of 370 (99.7%) of the available, template-intact specimens. We also used DNA sequencing to evaluate the accuracy of the realtime PCR methods using DNA extracts obtained from 1) 62 (15%) randomly selected operations classified as *M. ovipneumoniae*-infected, and 2) all enrichment broth specimens (N=180) in which *M. ovipneumoniae* was detected while estimating individual animal prevalence. All 62 randomly selected, pooled (operation-level) enrichment broths and all 180 individual ewe enrichment broths (composed of all samples in which *M. ovipneumoniae* was detected after 10 samples each were tested from 30 randomly selected operations) produced DNA sequences highly similar to *M. ovipneumoniae* GenBank entries. Together, these findings provide strong support for the accuracy of identification of *M. ovipneumoniae*-infected operations and individuals, and therefore, all results found to be positive by the original WADDL PCR test are defined as positive for these analyses. False negative misclassification of operations is another potential problem, and the limited numbers of ewes sampled per operation (N=16) predicts that there is a 5% or larger risk of false-negative misclassification of operations with an individual animal prevalence of 0.17 or lower (binary distribution). This risk of false negative classification may be the explanation for some or all of the 36 operations classified as *M. ovipneumoniae*-negative by PCR, but in which *M. ovipneumoniae* cELISA-reactive ewes were detected (Table S2).

3.3 *M. ovipneumoniae* presence and prevalence in surveyed operations

Of the operations contributing biological samples, 88.5% (401/453; 95% binomial confidence interval [85.2%, 91.3%]) tested PCR-positive for *M. ovipneumoniae*, and 85.3% (348/408; 95% confidence interval [81.5%, 88.6%]) showed signs of exposure to *M. ovipneumoniae* under the cELISA antibody assessment. *M. ovipneumoniae* prevalence varied substantially among the 62 size-stratified operations selected for individual-level testing (Figure S2). The median prevalence among those operations was 60%, though that figure should not be regarded as widely representative, given its basis on a stratified sample intended to cover a range of covariate values.

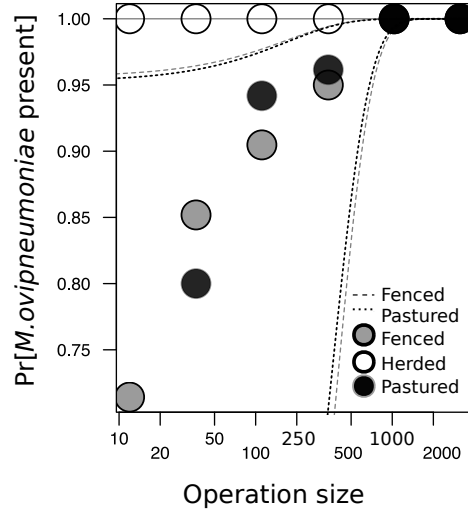


Figure 3: *M. ovipneumoniae* risk factors. Points show empirical proportions of operations containing PCR-detectable *Mycoplasma ovipneumoniae* infection, grouped by operation size and management type. Shaded regions show 95% credible intervals for the probability of *M. ovipneumoniae* detection in an operation, in a model that also accounted for operation size and management type. The dotted interval is for Pastured operations; the dashed interval is for Fenced (intervals overlap extensively). No interval could be calculated for operations with herded management, since all herded operations in this study showed active *M. ovipneumoniae* infections.

Table 1: USDA NAHMS Sheep 2011 survey coefficient estimates from a model of *M. ovipneumoniae* presence as a function of operation size, management type (baseline = Fenced), contact score, disease burden score, and antibiotic use score. This model was fit using the 408 operations that contributed biological samples, and where management was herded, pastured, or fenced.

Variable	Coefficient estimate	Standard error	Test statistic (p-value)
(Intercept)	0.754	0.406	1.855 (0.064)
management = Herded	1.768	1.469	1.203 (0.229)
management = Pastured	0.008	0.549	-0.515 (0.607)
operation size	0.005	0.002	2.819 (0.005)
contact score	0.072	0.062	1.722 (0.085)
disease burden score	-0.004	0.120	-0.029 (0.977)
antibiotic use score	0.379	0.295	1.281 (0.200)

3.4 *M. ovipneumoniae*-associated risk factors

All 47 herded operations tested PCR-positive for *M. ovipneumoniae*, as did 237 of 277 (86%) pastured operations, and 83 of 94 (88%) fenced operations. The median operation size of *M. ovipneumoniae*-positive operations was significantly larger (median size = 171 ewes) than negative operations (median size = 70 ewes, Wilcoxon p-value < 0.0001; Figure 3).

Biosecurity risk scores were marginally significantly higher in operations testing PCR-positive for *M. ovipneumoniae* than in operations testing negative ($W = 1503.5$, p-value = 0.069). There was evidence that PCR-positive operations were also subject to higher overall disease burden than negative operations (Wilcoxon rank-sum test $W = 7708.5$, p-value = 0.013). We found no significant association between antibiotic use and *M. ovipneumoniae* presence (Wilcoxon rank-sum test $W = 307.5$, p-value = 0.599).

In a logistic regression of *M. ovipneumoniae* presence as a function of the five postulated risk factors, only operation size and contact score retained their significance, and contact score only marginally so (Table 1).

The best explanatory model of *M. ovipneumoniae* prevalence within an operation for the 62 PCR-positive, individually sampled operations included only a single predictor, management type (AIC = 27.5). Models that also included

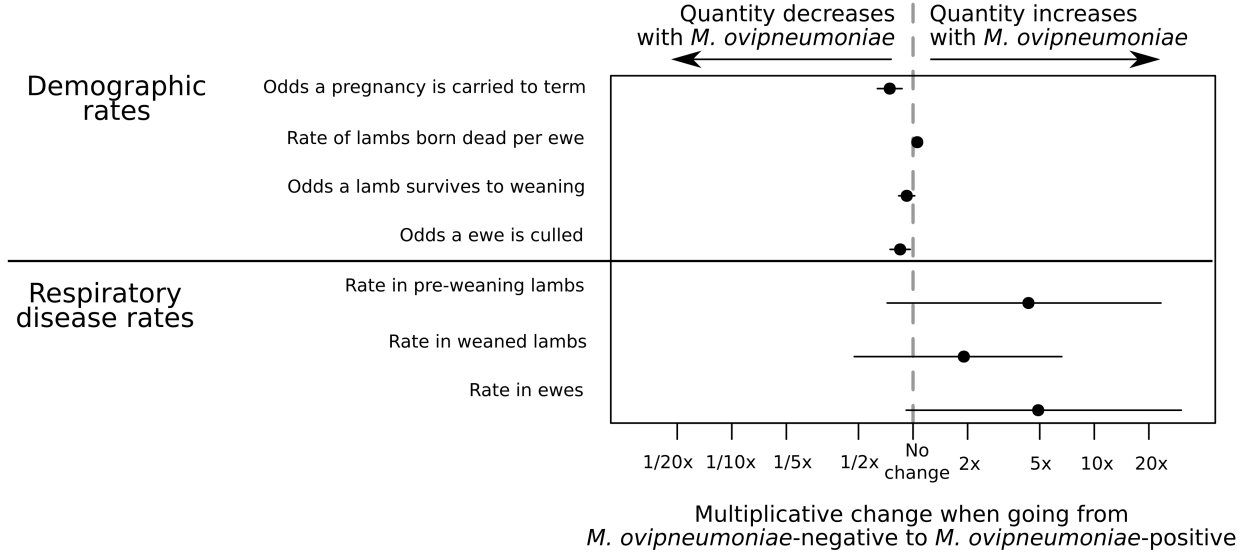


Figure 4: **Associations between *M. ovipneumoniae* presence and production rates.** Estimated changes in vital rates and rates of reported respiratory disease incidence in operations with PCR-detectable *Mycoplasma ovipneumoniae*, relative to uninfected operations. The x-axis shows the multiplicative change in each rate in the presence of *M. ovipneumoniae*, relative to the rate in the absence of *M. ovipneumoniae*. For instance, an interval extending from 1.2 to 2.6 would indicate that the corresponding rate is expected to be between 1.2 and 2.6 times higher in *M. ovipneumoniae*-positive flocks than in *M. ovipneumoniae*-negative flocks.

antibiotic use score (AIC = 28.4), operation size (AIC = 28.6), and disease burden score (AIC = 28.9) in addition to management type were competitive with management type alone, but these multivariate models were not selected because the individual effects were not significant ($p > 0.05$ delta-AIC > 2, Table S3).

3.5 Associations between animal health measures and *M. ovipneumoniae* presence

M. ovipneumoniae-infected operations often exhibited significantly less-productive vital rates than comparable operations without *M. ovipneumoniae*, after accounting for differences in management type and operation size (Figure 4). Birth rates among pregnant ewes were lower in *M. ovipneumoniae*-positive operations than in *M. ovipneumoniae*-negative operations (posterior mode for $e^\beta = 0.744$, 95% credible interval = [0.646, 0.870]; pseudo R-squared = 0.142, Table S4). Number of lambs born per pregnant ewe was higher in *M. ovipneumoniae*-positive operations than in *M. ovipneumoniae*-negative operations (posterior mode for $e^\beta = 1.055$, 95% credible interval = [1.024, 1.088]; pseudo R-squared = 0.082, Table S5), but *M. ovipneumoniae*-positive operations also had a higher proportion of lambs born dead than *M. ovipneumoniae*-negative operations (median proportion of full-term lambs born dead = 0.052 in *M. ovipneumoniae*-positive operations vs. 0.037 in *M. ovipneumoniae*-negative operations). Lamb survival to weaning was also lower, though not significantly so, in the presence of *M. ovipneumoniae* (posterior mode for $e^\beta = 0.922$, 95% credible interval = [0.833, 1.020]; pseudo R-squared = 0.171, Table S6). Overall ewe culling rates were higher in the presence of *M. ovipneumoniae* than in *M. ovipneumoniae*'s absence (posterior mode for $e^\beta = 1.178$, 95% credible interval = [1.036, 1.339]; pseudo R-squared = 0.147, Table S7). *M. ovipneumoniae* was associated with marginally higher lamb weaning weights (posterior mode for $\beta = 4.890$, 95% credible interval = [-0.953, 10.733], $p = 0.102$, Table S8, though this effect was not statistically significant at a 0.05 significance level, and may be due to unmodeled differences in twinning rates between *M. ovipneumoniae*-positive and -negative operations.

The data analyzed here did not provide conclusive evidence that culling rates increased for ewes, pre-weaning lamb, or weaned lambs at *M. ovipneumoniae*-positive operations over rates at *M. ovipneumoniae*-negative operations, after accounting for management type and operation size (posterior mode for $e^\beta = 4.903$, 95% credible interval = [0.917, 30.204], Table S9; pre-weaning lambs' posterior mode for $e^\beta = 4.333$, 95% credible interval = [0.720, 23.313], Table S10; weaned lambs' posterior mode for $e^\beta = 1.907$, 95% credible interval = [0.474, 6.615], Table S11).

3.6 Associations between vital rates and *M. ovipneumoniae* prevalence

WADDL and TEB's laboratory conducted additional testing on a subsample of 62 *M. ovipneumoniae*-positive operations to determine individual ewe prevalence. Higher prevalence (number of individuals testing PCR-positive for *M. ovipneumoniae* out of ten) was associated with lower full-term birth rates among pregnant ewes (posterior mode for $e^\beta = 0.709$, 95% credible interval = [0.572, 0.878], $p = 0.002$, pseudo R-squared = 0.877, Table S12) in models that also accounted for operation size and management type. Increased prevalence was associated with a decreased number of lambs born per ewe (posterior mode for $e^\beta = 0.758$, 95% credible interval = [0.726, 0.792], $p < 0.0001$, Table S13). Increased *M. ovipneumoniae* prevalence was associated with lower lamb survival to weaning among lambs born alive (posterior mode for $e^\beta = 0.455$, 95% credible interval = [0.391, 0.530], $p < 0.0001$, pseudo R-squared = 0.159, Table S14). Ewe culling rates increased significantly with increasing *M. ovipneumoniae* prevalence (posterior mode for $e^\beta = 1.722$, 95% credible interval = [1.484, 1.998], $p < 0.0001$, pseudo R-squared = 0.092, Table S15). There was no relationship between lamb weight at weaning and *M. ovipneumoniae* prevalence (posterior mode for $\beta = 0.784$; 95% credible interval = [-0.532, 2.101], $p = 0.908$, Table S16). Predicted changes in vital rates in response to the changes in *M. ovipneumoniae* prevalence reported in Table 2 are based on these models.

3.7 Consequences of *M. ovipneumoniae* infection on operation productivity

To evaluate the impact of infection on production, we compared two median-sized operations of 155 ewes each, one infected with *M. ovipneumoniae* and one uninfected. Of those ewes, we would expect 10.2% (15.8) to be culled in the *M. ovipneumoniae*-positive operation, and 9.0% (14.0) to be culled in the *M. ovipneumoniae*-negative operation, leaving 139.2 and 141.0 ewes, respectively. If all surviving ewes are bred at both operations, then the NAHMS data suggests that 141.0 (100%) of the ewes from *M. ovipneumoniae*-negative operation would deliver full-term lambs, but only 133.0 (95.4%) of the ewes from the *M. ovipneumoniae*-positive operation would do so. In the *M. ovipneumoniae*-positive operation, we would expect each bred ewe to produce 1.53 live lambs, for a total of 203.5 lambs born alive. In the *M. ovipneumoniae*-negative operation, that rate drops to 1.47 live lambs per ewe, for an expected total of 207.3 lambs born alive. Of those lambs, we would then expect 198.4 (95.7%) to survive to weaning at the *M. ovipneumoniae*-negative operation, but only 189.9 (93.3%) to survive to weaning at the *M. ovipneumoniae*-positive operation. Therefore, in aggregate, we would anticipate a reduction of roughly 8.5 lambs (4.3%) for an *M. ovipneumoniae*-positive operation, in comparison with an *M. ovipneumoniae*-negative but otherwise identical operation. If we finally assume that lambs can be sold for \$75-\$150 per head, this translates to an annual *M. ovipneumoniae*-imposed cost of \$637-\$1,275 for a median-sized flock.

4 Discussion

4.1 Conclusions

Evidence of *M. ovipneumoniae* infection was detected over 85% of the herded, pastured, and fenced domestic sheep operations that contributed biological samples to the first comprehensive survey of *Mycoplasma ovipneumoniae* in U.S. domestic sheep operations. Prevalences varied widely among the operations that received individual-level follow-up testing, from a single infected animal out of 10 sampled to universal infection in 10 sampled individuals. *M. ovipneumoniae*'s near ubiquity across domestic sheep operations means that even if infection has very small economic consequences at the individual level, those consequences likely accumulate over large numbers of animals and operations. This potentially imposes a heavy but unrecognized disease cost on domestic sheep production in the United States that contrasts with rare-but-devastating diseases like Scrapie which have the potential to accumulate high and visible economic burdens over a relatively small number of animals and operations (e.g., [33]). While there are relatively few survey studies of *M. ovipneumoniae* presence and prevalence in domestic sheep, our findings are on the same order as those of [2]. Those authors reported *M. ovipneumoniae* presence (as determined by PCR) in 8 of 11 tested flocks in the United Kingdom. In their case, however, seven of those flocks had a reported history of respiratory disease, and it is not entirely clear if flocks were chosen for testing on the basis of that history. Akwuobu et al. [34] reported 25 *M. ovipneumoniae* detections among 172 sample sheep from small ruminant operations in Nigeria, and Amin et al. [35] found a prevalence of 16.5% across 1047 samples from 139 flocks in Baluchistan, Pakistan. These individual-level prevalences are lower than those from our stratified sample, but production conditions are quite different.

Our estimate of *M. ovipneumoniae*'s burden on domestic sheep operations is subject to a number of sources of variation. In particular, we used vital rate estimates at the population level to estimate disease effects that actually operate at the individual level. Therefore, our individual-level burden estimates may be biased low, and are subject to additional variation associated with flock-level prevalences. *M. ovipneumoniae*'s burden was distributed across a variety of vital rates, as is true for other chronic ovine diseases like Johne's disease [36]. While the social costs of *M. ovipneumoniae*

are likely much lower than those of diseases with zoonotic potential, *M. ovipneumoniae* is nevertheless associated with substantial costs for production. Our estimate of *M. ovipneumoniae* burden may also be somewhat confounded by the overall higher disease burden exhibited by *M. ovipneumoniae*-positive operations. Further work is needed to separate *M. ovipneumoniae* effects from those of other pathogens. Nevertheless, losses of this magnitude may merit additional inquiry into management practices that could mitigate *M. ovipneumoniae* presence and prevalence per se. The ubiquity of *M. ovipneumoniae* infection in domestic sheep currently is a major challenge for control. Exploration into novel approaches that might be more widely applicable and cost-effective is a worth avenue for future work.

We were surprised by the positive effect of *M. ovipneumoniae* presence on the number of lambs born per ewe. Our analysis showed apparently divergent effects: while *M. ovipneumoniae* presence (binary) was associated with more lambs born per bred ewe, increased *M. ovipneumoniae* prevalence (continuous, and only among *M. ovipneumoniae*-positive operations) was associated with fewer lambs born per bred ewe. It is possible that some unmeasured factor associated with more lambs born per ewe was more common among *M. ovipneumoniae*-positive operations (potentially due to divergent management practices in positive and negative operations). When prevalence (only measured among infected operations) was considered, however, the coarse factor separating positive and negative operations may have been removed, allowing the weaker, yet still present *M. ovipneumoniae*-related vital rate shift to emerge. One plausible explanatory factor is mode of breeding. Smaller operations, which were also more likely to be *M. ovipneumoniae*-negative, may have used less reliable methods to determine how many of their ewes were bred than their larger counterparts. If small operators tended to over-estimate their numbers of “bred” ewes relative to large operators, that would inflate their denominator values in estimated number of lambs born per bred ewes, producing the apparently lower multiple birth rate reported here. Regardless of whether this is in fact the case, all vital rates presented here, and especially the multiple birth rate estimate, should be the topic of future investigation.

We saw significantly higher weaning weights in flocks that had any *M. ovipneumoniae* presence than in flocks where no *M. ovipneumoniae* was detected (i.e., a categorical variable where 1 = *M. ovipneumoniae*-positive and 0 = *M. ovipneumoniae*-negative), though this effect might be partially explained by differences in twinning rates between *M. ovipneumoniae*-positive and *M. ovipneumoniae*-negative operations. When we limited our inquiry to just *M. ovipneumoniae*-positive flocks, we saw no effect of prevalence on weaning weights. The direction of the effect was still positive (which is to say, higher prevalence was associated with higher weaning weight), but the standard error on that estimate was high enough that the estimated effect could have easily been due to sampling variation.

Operation size and management type were strongly related in the NAHMS dataset. This relationship did not have a strong deleterious effect on our model results; however, standard error estimates associated with size and operation effects did not change dramatically when models included only one or both variables as predictors. We present results from models containing both risk factors here, with the understanding that in practice, some operation sizes are only feasible under certain management types.

4.2 Project strengths and limitations

4.2.1 Scope of Inference

The NAHMS data presented here represent health patterns in operations with 20 or more ewes as of 2011, included in one of the 22 sampled states ([10] pg. 145). These are the strongest data available for studying prevalence of under-reported pathogens in domestic sheep throughout the United States, since invitation to participate in the NAHMS survey is determined through a rigorously designed, state-of-the-art sampling protocol. However, any sampling protocol that incorporates some element of participant choice (in this case, the invitees’ ability to decline the invitation to participate) can potentially depart from its intended stratification. As a consequence, and to avoid compounding even small biases due to participant choice, the sample data were not weighted back to the population totals in the present analysis.

Although our results are correlative in nature, our findings nonetheless suggest that *M. ovipneumoniae* may pose substantial yet under-recognized costs for domestic sheep production in the United States. Additional efforts to limit *M. ovipneumoniae* infection in domestic sheep operations could be of financial benefit to operators, and cost-effective methods to reduce infection burden should be explored.

4.2.2 Confounding with other respiratory diseases, and with region

The relationship between *M. ovipneumoniae* presence and vital rates and disease burden may be somewhat confounded by the generally higher overall disease burden exhibited by *M. ovipneumoniae*-positive operations. Further work is needed to separate *M. ovipneumoniae* effects from those of other pathogens, in particular ovine progressive pneumonia. Nevertheless, losses of this magnitude merit some additional inquiry about management practices that could mitigate *M. ovipneumoniae* presence and prevalence, along with those of other respiratory pathogens.

M. ovipneumoniae diagnostic testing continues to be an area of active refinement. The method initially used to test these samples is now known to cross-react on occasion with another Mycoplasma-like organism, but the re-testing we conducted using a modified test that eliminates that cross-reaction, together with the sequence confirmation of 180 test-positive samples from 30 randomly selected operations, confirms the lack of significant cross-reaction in the original testing for this study. Nevertheless, there is some possibility that additional refinement or recategorization of Mycoplasma spp. might produce different testing results in the future.

We omitted region information from our analysis, and this may have some consequences on our inferences. Region was confounded with management type and operation size, which were important predictors of *M. ovipneumoniae* prevalence and presence. We could not effectively separate region effects from management type effects within this dataset, so here we attribute the *M. ovipneumoniae* risk to management type. However, geographic effects should be addressed explicitly in follow-up work, if possible.

4.2.3 Within-operation sampling

We had limited information on how individual animals were selected for sampling within a flock. If *M. ovipneumoniae* transmission occurs predominantly within groups, and all sampled individuals were from the same group, then there might be a lack of independence among animals sampled within a particular operation. This correlation is unlikely to have introduced substantial bias into inferences based on *M. ovipneumoniae* presence, but could have produced biased results related to prevalence. In an effort to limit the role of that potential bias here, we chose not to emphasize overall prevalence rates, and focused instead on relationships between prevalence and various vital rates. Nevertheless, clustering should be explored in future investigations.

An additional issue associated with sampling is sample size within operations. These small sample sizes limited the precision of estimated relationships between *M. ovipneumoniae* prevalence and vital rates. For this reason, we primarily focused on the direction of estimated effects, and base our cost projections on an aggregate across all estimated vital rate relationships. Larger sample sizes from a carefully chosen set of focal operations would add valuable clarity to both the prevalence-vital rate relationships, and the cost projections.

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CONTAGIOUS ECTHYMA IN BIGHORN SHEEP AND MOUNTAIN GOAT IN WESTERN CANADA 1

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CONTAGIOUS ECTHYMA IN BIGHORN SHEEP AND MOUNTAIN GOAT IN WESTERN CANADA^[1]

W. M. SAMUEL,^[2] G. A. CHALMERS,^[3] J. G. STELFOX,^[4] A. LOEWEN,^[5] and J. J. THOMSEN^[6]

Abstract: Contagious ecthyma (CE) is reported in bighorn sheep (*Ovis c. canadensis*) from several national parks in western Canada and in mountain goat (*Oreamnos americanus*) from Kootenay National Park, British Columbia. (This is the first report of CE in mountain goat.) Diagnosis was based on clinical signs, histopathology, transmission experiments and the demonstration of a poxvirus with the electron microscope. The infection was transmitted from wild to domestic goat, but not to domestic sheep.

Most infections, some of them severe, were found in lambs and kids. Clinical signs of disease were similar to those seen in domestic sheep and goats. General body condition was poor and animals had difficulty feeding normally.

All infected herds had prolonged contact with areas where salt was provided artificially (i.e., salt blocks, highways and campgrounds). Fewer infected sheep were observed annually when salt blocks were removed from Jasper National Park.

INTRODUCTION

Contagious ecthyma (CE) is a viral disease of domestic sheep and goats from many countries and is known by a variety of descriptive terms including sore mouth, contagious pustular dermatitis, scabby mouth, infectious labial dermatitis, and orf.⁴ The clinical disease is characterized by proliferative, crusted and sometimes pustular lesions of the lips, muzzle and occasionally the udder, feet and vulva.⁶ Little is known about CE in wild animals; it has been reported only from bighorn sheep, chamois, thar and steinbok.⁶ Connell² and Blood¹ reviewed the history of CE to 1965 in bighorn sheep from four national parks in western Canada; no mortality was attributed directly to CE, but several cases of the disease were observed in lambs. This report summarizes recent developments concerning CE in bighorn sheep (*Ovis c.*

canadensis) from western Canada and documents the disease in mountain goats (*Oreamnos americanus*).

HISTORY

CE has been observed in bighorn sheep from western Canada since 1953.² Sixteen infected sheep comprising 11 lambs and five ewes, were collected from Jasper, Banff, Kootenay and Waterton Lakes National Parks since 1953¹ (Stelfox, unpub.). Most animals were in debilitated condition, with severe labial lesions. Many other sheep, particularly lambs, were observed with lesions suggestive of CE.

Since 1959, 58 suspected cases which includes 49 lambs have been observed in Jasper National Park. The number of suspected cases remained low (≤ 3) each year until 1966, when 15 of 76 sheep in

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the Mile 3 band (see Fig. 1) and two other sheep from other bands were infected. In the Disaster band which has been observed closely since 1968, 51 of approximately 80 sheep were examined in February, 1968; one of 14 lambs was infected. Two of four lambs in a group of 12 were infected in September, 1970. One of eight lambs in a band of 42 sheep was infected in December, 1970. The number of presumptive cases observed annually from 1967 to June 7, 1973, in Jasper was 6, 4, 8, 4, 4, 2, and 0 respectively.

Of the 58 cases observed in Jasper National Park, 52 were seen from September to December, five from January to March and one infected lamb was observed in mid-July. None were observed in August and 10 in September.

In all cases observed in the national parks,¹ sheep have had prolonged contact with areas where salt was provided artificially (i.e., salt blocks, highways and campgrounds).

Lesions suggestive of CE have been observed in bighorn sheep east of Banff National Park,¹ in the Sheep River area in 1972 and 1973 (Horejsi, pers. comm.) (Fig. 1), and in Montana.¹ Six of 19 lambs were infected in a band of 43 sheep trapped from January to March, 1972, at Sheep River. In June-July 1972, three infected lambs were observed; during the winter, 1973, eight of 19 lambs were infected. None of the infections was considered severe (Horejsi, pers. comm.). Sheep visit artificial sources of salt between September and May of each year in the Sheep River area.

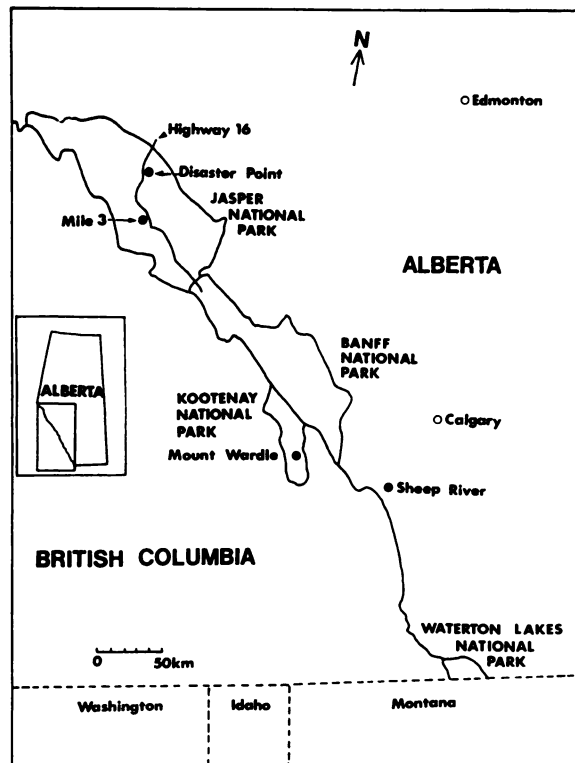


FIGURE 1. Location of western National Parks and several infected bands of bighorn sheep and mountain goat.

CE has been observed in goats from Mount Wardle since 1974 (Sime, pers. comm.). Four adults, two males and two females, were found moribund in January 1947 with severe lesions on the lips and muzzle. All animals died within 1 month of capture. Approximately 90 adults produced 18 kids on Mount Wardle in 1967, and at least six kids were observed with clinical signs of the disease (DeBock and Winkler, pers. comm.). Three of the kids with severe lesions of the lips and muzzle were found moribund in July. A 9-month old male with severe labial lesions was collected on Mount Wardle in February 1972; it weighed 12 kg. There were no reports of CE infection in 1973. Four of five mineral licks used by goats on their winter range are semi-natural.³ No infected sheep have been observed on Mount Wardle (Stelfox, unpub.), but CE-infected sheep have been collected from other parts of Kootenay National Park.¹

CASE REPORT — SHEEP

Two emaciated animals, a ewe and lamb, with severe lesions of the muzzle and lips, were observed and subsequently killed in Waterton Lakes National Park (Fig. 1) in April, 1971. The ewe was emaciated and weighed only 26 kg.

Gross examination of the lamb revealed numerous irregular, confluent, raised, crusted, dark brown to black lesions located along the margins of the lips including the commissures, and on the muzzle and nose. Most lesions were dry, but pustules were also present. Lesions were particularly severe in the ewe (Figs. 2-3) and were characterized by markedly thickened, edematous lips covered by reddish-brown, fissured, crusted proliferative epithelium (Fig. 2). Several pale, circumscribed, raised lesions of various diameters were observed on the dorsum of the tongue; smaller red papilloma-like proliferations, some of them peridunculated, were evident along the anterior leading edge. Numerous small pink proliferative lesions were observed clustered on the gingival mucosa at the base of the incisiform teeth

(Fig. 3); blackened proliferations could also be found on the dental pad, hard palate and at the base of the molars.

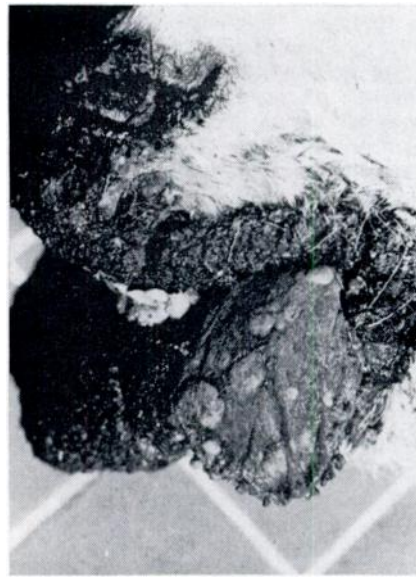


FIGURE 2. Contagious ecthyma in a bighorn sheep. Lesions on the nose, lips and tongue.



FIGURE 3. Contagious ecthyma in a bighorn sheep. Lesions on the gingival mucosa.

CASE REPORT — GOAT

A severely infected moribund mountain goat kid (Fig. 4) was found by park personnel at the base of Mount Wardle in Kootenay National Park (Fig. 1) on March 1, 1972, and was transported to the University of Alberta on March 9, 1972. The kid weighed 13.4 kg on arrival and improved during its second week, following treatment for suspected nutritional myopathy. Although appetite was good, prehension and mastication were difficult and the animal's condition continued to deteriorate. At the time of death on April 3, 1972, the kid weighed 11.5 kg.

Dry, brown, proliferative lesions were prominent on the markedly edematous hyperemic lips, but diminished in severity toward the commissures (Fig. 4). Lesions of the oral cavity were moist, reddish-brown and in certain sites, intensely hyperemic. A large proliferative mass on the gingiva immediately posterior to the incisiform teeth apparently interfered with the normal placement of the tongue.

Examination of the remainder of the carcass revealed numerous pulmonary

abscesses along the ventral borders of the diaphragmatic lobes, with extensive fibrous adhesions to the ribs and diaphragm. As well, abscesses were observed on the pericardial sac and in the abdominal cavity, with many fibrous adhesions between the diaphragm and the rumen. Bacteriologic examinations of pulmonary abscesses yielded *Corynebacterium pyogenes* and *Pasteurella multocida*. The following helminths were found: 790 *Ostertagia occidentalis* and *Marshallagia marshalli* (abomasum); 249 *Nematodirus maculosus*, several *Thysanosoma actinioides*, many unidentified Anoplocephalidae (small intestine); 123 *Trichuris oreamnos* (caecum); and 3 *Protostrongylus rushi* (lungs).

HISTOPATHOLOGY

Selected oral and labial lesions were trimmed to 2-3 mm thickness, fixed in 10% neutral buffered formalin, processed in several changes of ethyl alcohol, xylene and paraffin, cut at 6 μ m and stained with haematoxylin and eosin (H & E).

Histopathologic lesions were similar in both the sheep and goat. Hyperplastic non-pigmented squamous epithelium, often thrown upward into folds and long spike-like projections with marked hyperkeratosis, some parakeratosis and microabscesses in the keratinized layer, characterized the lesions. Edema was prominent at the tips of epithelial folds, and in the keratinized layer, pools of serous fluid separated by thin keratin strands had a characteristic multilocular appearance. Long interconnecting rete pegs extended into the dermis, in which the proliferating connective tissue was canalized by dilated erythrocyte-filled capillaries. Inflammatory cells in the dermis consisted mainly of dense accumulations of lymphocytes, with lesser numbers of plasmacytes, neutrophils and some eosinophils.

Numerous swollen, vacuolated epithelial cells, best appreciated in the immediate vicinity of hair follicles, each contained one or more oval or round eosinophilic cytoplasmic inclusions.



FIGURE 4. Lesions of contagious ecthyma on the lips of a mountain goat.

VIRAL ISOLATION AND TRANSMISSION ATTEMPTS

Lesions were removed from the lip of the mountain goat and stored in refrigerated sterile buffered glycerine. Tissue was washed 3 times in saline and ground in a mortar to prepare a 10% suspension based on wet tissue weight. Transmission was attempted by spreading the suspension on a shaved, lightly scarified area of the flanks of two domestic sheep and one domestic goat. Typical vesicles and pustules characteristic of CE began to appear at the site of inoculation on the goat at 21 days. Lesions did not appear on the sheep. Typical CE viral particles⁷ were demonstrated by electron microscopy, using negative staining with 2% pH 7.0 phosphotungstate, from extracts of swabs from the wild and domestic goats. The lesions on the domestic goat began to heal 30-35 days after inoculation; the inoculation site was clear by 40 days. CE has been transmitted successfully from bighorn to domestic sheep previously.^{1,2}

DISCUSSION

CE has been reported rarely from animals other than domestic sheep and goat. The present data and those of Blood¹ indicate an important large focus of CE in bighorn sheep in British Columbia and Alberta. The disease is apparently restricted to certain mountain goat populations, having been reported only from Mount Wardle in Kootenay National Park, British Columbia. Many bands of uninfected goats have been observed in and near western Canadian national parks (Stelfox, Loewen, unpub.,

and Cowan, Hebert and others, pers. comm.).

Several sheep and goats severely infected with CE have been found dead or moribund. It is evident that prehension and mastication are altered if the lips, muzzle and buccal membranes are severely affected. These observations suggest strongly that CE, possibly in combination with high numbers of helminths (Samuel, unpub.), bacterial infections and a low plane of nutrition, is an important mortality factor in certain bands of wild sheep and goats. In animals with mild infections, mortality due to CE *per se* is probably negligible. Horejsi (pers. comm.) noted no difference between the feeding behavior of infected and uninfected lambs at Sheep River.

The role of artificial sources of salt in the epidemiology of CE is unclear. For many years, salt blocks were placed strategically in the parks to concentrate sheep and other ungulates for observation by tourists. A dangerous zoonotic component of this policy was the observation of children breaking off and licking pieces from salt blocks used by infected bands of sheep (Stelfox, unpub.). This distribution of salt, predominately along the major road in Jasper Park, was discontinued between 1966 and 1970. However, sheep continued to return to the lick areas and eat large quantities of soil or to receive feed from tourists. Bands at Mile 3 and Disaster obtain limited quantities of salt from tourists and highway sources. At least one uninfected band of 60 sheep near the Jasper-Banff boundary has had access to block salt in the past and to heavy highway salting at the present time.

Acknowledgments

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RECOMMENDATIONS FOR
**Domestic Sheep and Goat Management
in Wild Sheep Habitat**



Prepared by the
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Executive Summary

Executive Summary

Although the risk of disease transmission from domestic sheep or goats to wild sheep is widely recognized, a unified set of management recommendations for minimizing this risk has not been adopted by responsible agencies. These Western Association of Fish and Wildlife Agencies (WAFWA) recommendations were produced to help state, provincial, and territorial wild sheep managers, federal/crown land management agencies, private landowners and others take appropriate steps to eliminate range overlap, and thereby, reduce opportunities for transmission of pathogens to wild sheep.

Transmission of *Mannheimia haemolytica* from domestic sheep to bighorn sheep was irrefutably demonstrated by Lawrence et al. (2010) and provides justification sufficient for preventing range overlap and potential association of domestic sheep and goats with wild sheep. The higher the

conservation value of a wild sheep population (e.g., federally or state listed, “sensitive species” status, native herds, transplant source stock, herds in areas with no history of domestic livestock presence), the more aggressive and comprehensive wild sheep and domestic sheep or goat separation management strategies should be.

Practical solutions will be difficult, if not impossible to achieve until the risk of disease transmission from domestic sheep or goats to wild sheep is acknowledged by those responsible for wildlife and agricultural management. All parties benefit when risk is assessed and actively managed to minimize the potential for transmission of pathogens. The recommendations contained within this report are intended to help achieve that objective to benefit all sectors and are summarized as follows:

WAFWA agencies should:

(1) assess wild sheep conservation value/status and complete risk assessments of interspecies contact in a meta-population context; (2) remove wild sheep that have likely associated with domestic sheep or goats and develop a policy to promptly respond to wild sheep wandering from occupied wild sheep ranges; (3) thoroughly explore demographic consequences of translocations and conduct appropriate analyses of habitat suitability and risk of disease transfer prior to implementing any translocations; (4) coordinate with other agencies, land owners and stakeholders regarding management of domestic sheep or goats on or near ranges occupied by wild sheep; (5) fully consider the risk of disease transmission when issuing or commenting on permits/regulations associated with private lands used for domestic production; and (6) develop educational materials and outreach programs to interpret the risk of association between wild sheep and domestic sheep or goats.

Land management agencies should:

(1) reduce risk of association by eliminating overlap of domestic sheep or goat allotments or grazing permits/tenures within wild sheep habitat; (2) ensure that annual operating instructions or their equivalent include measures to minimize domestic association with wild sheep and confirm appropriate methods to remove stray domestic sheep or goats; and (3) manage wild sheep habitat to promote healthy populations in areas without domestic sheep or goats.

Wild sheep conservation organizations should:

(1) assist with educational/extension efforts to all parties; (2) negotiate alternatives and incentives for domestic sheep or goat grazers on public land to find alternatives to wild sheep habitat; and (3) advocate for and support research concerning disease and risk associated with domestic sheep and goats in proximity to wild sheep.



Photo by: David Wetzel (Texas Bighorn Society)



Photo by: Tom Carlsen (MDFWP)



Introduction

Domestic sheep and goat permittees/owners should:

(1) implement best management practices (BMPs) to prevent straying by domestic sheep or goats; and (2) establish protocols to respond to straying.

Private landowners should:

(1) educate themselves and work with wild sheep managers and advocates to support effective separation through a variety of site-specific mitigation measures; and (2) promptly report the potential or actual association between domestic sheep or goats and wild sheep.

Introduction

In January 2007, the Western Association of Fish and Wildlife Agencies (WAFWA), comprised of 23 state and provincial wildlife agencies from the western United States (U.S.) and western Canada, established a Wild Sheep Working Group (WSWG) to develop a report titled, “Recommendations for Domestic Sheep and Goat Management in Wild Sheep Habitat” (WAFWA 2007). Unanimously endorsed by WAFWA Directors in July 2007, that report provided recommendations to which state, provincial and federal agencies could tier their management actions. In August 2007, the report was forwarded to the heads of the U.S. Forest Service (USFS), Bureau of Land Management (BLM), National Park Service, U.S. Fish and Wildlife Service, Bureau of Reclamation, and Department of Defense. In July 2010, the report was revised (WAFWA 2010c) and has represented the official position of WAFWA on the management of domestic sheep and goats and wild sheep.

Scientific literature that has become available since July 2010 has been incorporated into this document to ensure that the recommendations contained herein remain current and robust, but the basic purpose, scope, and principles of the document remain unchanged. Additional editorial modifications are intended to improve the readability of the document. Information contained in this report is provided to assist BLM and USFS leadership with development of a unified policy addressing the grazing of domestic sheep or goats in wild sheep habitat on lands under the administration of those agencies. In addition, this document is intended to assist state, provincial, and territorial wild sheep managers, federal/crown land management agencies, private landowners and others take appropriate steps to eliminate range overlap, and thereby, reduce opportunities for transmission of pathogens to wild sheep. This revision was approved by the WAFWA Directors March 29, 2012, and supersedes all previous versions.

In this paper we do not review and synthesize all available literature or evidence pertaining to the issue of disease transmission among bighorn sheep and domestic sheep and goats. We do, however, include relevant citations, results,



Photo by: Earl Nottingham (TPWD)



Photo by: Dr. Peri Wolff (NDOW)

literature, or analyses published since completion of our previous reports (WAFWA 2007, 2010c). We provide reasonable and logical recommendations based on the best available information to help achieve effective separation between wild sheep and domestic sheep or goats. We recognize it is impossible to achieve zero risk of contact or disease transmission; however, we also recognize there are many ways to reduce the probability of association between these species and, thereby, lower the overall risk of epizootics occurring in populations of wild sheep.

Background



Photo by: David Wetzel (Texas Bighorn Society)



Photo by: Mike Pittman (TPWD)



Photo by: Brett Wiedmann (NDGFD)

Background

Throughout substantial portions of their range, bighorn sheep (*Ovis canadensis*) experience periods when populations are depressed; those episodes generally are associated with epizootics of respiratory disease (Ryder et al.1994). Diseases have contributed to the decline of bighorn sheep populations in much of western North America (Beecham et al. 2007, CAST 2008) and many native herds declined to less than 10% of historical size. According to historical accounts, such declines coincided with the advent of domestic livestock grazing on ranges occupied by bighorn sheep (Warren 1910, Grinnell 1928, Schillinger 1937, Honess and Frost 1942, CAST 2008). Epizootics among native bighorn herds were reported in various locations following European settlement and establishment of domestic livestock grazing throughout the central and southern Rocky Mountains. These observations may reflect the introduction of novel bacterial pathogens (including some strains of *Pasteurella [Mannheimia]* spp.) to naïve bighorn populations beginning in the late 1800s (Grinnell 1928, Skinner 1928, Marsh 1938, Honess and Frost 1942, Miller 2001).

Over the past 30 years, increasing evidence has underscored the potential risk of disease transmission from domestic sheep or goats to wild sheep (McQuivey 1978, Hunt 1980, Jessup 1982, Foreyt and Jessup 1982, Goodson 1982, Onderka and Wishart 1984, Jessup 1985, Black et al.1988, Coggins 1988, Festa-Bianchet 1988, Onderka and Wishart 1988, Onderka et al.1988, Schwantje 1988, Callan et al.1991, Coggins and Matthews 1992, Foreyt 1994, Foreyt et al. 1994, Cassirer et al.1996, Foreyt and Lagerquist 1996, Martin et al. 1996, Coggins 2002, Rudolph et al. 2003, Jenkins et al. 2007, Rudolph et al. 2007, George et al. 2008, Jeffress 2008, Lawrence et al. 2010).

Moreover, a number of recent risk assessments and reviews (Beecham et al. 2007, CAST 2008, Baumer et al. 2009, USAHA 2009, WAFWA 2009, Croft et al. 2010, USDA Forest Service 2010a, b; Wehausen et al. 2011), conservation management strategies or plans (Colorado Division of Wildlife 2009, Montana Department of Fish, Wildlife, and Parks 2009), modeling exercises (Clifford et al. 2009, Cahn et al. 2011), and many wildlife biologists and wildlife veterinarians (Gross et al. 2000, Singer et al. 2000, Dubay et al. 2002, Epps et al. 2004, Garde et al. 2005, Jansen et al. 2006, Foreyt et al. 2009) have focused on risks associated with contact between wild sheep and domestic sheep or goats. Many of the aforementioned investigators and participants in workshops conducted throughout the western US (California, Arizona, Utah, and Idaho),

have recommended temporal or spatial separation of domestic sheep or goats from wild sheep to reduce the potential for disease in the latter.

Disease Transmission

Although domestic animals have been selected for their ability to live at high densities and for their resilience to infectious diseases (Diamond 1997), two-way transmission of certain diseases (e.g., paratuberculosis, some enteric pathogens and parasites) between wild sheep and domestic sheep or goats in shared habitats can occur (Garde et al. 2005). However, the most important and ecologically significant transmission in this context is from domestic sheep or goats to wild sheep.

Winter 2009-2010 bighorn sheep pneumonia die-offs (totaling an estimated 880 bighorns) in Montana, Nevada, Washington, Utah, and Wyoming have reduced bighorn numbers in at least 9 herds, either through direct mortality or agency removal (i.e., “culling”) of bighorn sheep exhibiting symptoms of respiratory infections (Edwards et al. 2010, WAFWA 2010b). Domestic sheep and goats were known to occur within or near occupied bighorn sheep ranges and within normal bighorn movement zones, and association between wild sheep and domestic sheep or goats is known to have preceded at least one of these die-offs, was likely in 2 others, and was possible in 4 more (WAFWA 2010b).

Die-offs of wild sheep populations and individual animals have occurred in the absence of reported association with domestic sheep or goats (Aune et al. 1998, UC-Davis 2007). However, when contact between wild sheep and domestic sheep or goats has been documented, the pattern and severity of die-off is typically greater than when otherwise is the case (Onderka and Wishart 1984, Martin et al. 1996, Aune et al. 1998, George et al. 2008).

It is generally acknowledged (Garde et al. 2005, CAST 2008) that thimhorn sheep (*Ovis dalli* spp.) in Alaska and northwestern Canada are likely naïve to exposure to many organisms commonly carried by domestic species, compared to wild sheep occurring in southern Canada and the continental U.S. Until this is confirmed and the effects of exposure to infectious organisms are clearly understood, it is essential that no association occurs between thimhorn sheep and domestic sheep or goats.



Photo by: Donny Martorello (WDFW)



Photo by: Ernie Finch

Effective Separation



Photo by: John Kanta (SDGFP)



Photo by: Ted Borda (Borda Land & Sheep Company)



Photo by: Ted Borda (Borda Land & Sheep Company)

Effective Separation

WAFWA defines “Effective Separation” as spatial or temporal separation between wild sheep and domestic sheep or goats to minimize the potential for association and the probability of transmission of diseases between species. WAFWA advocates that effective separation should be a primary management goal of state, provincial, territorial and federal agencies responsible for the conservation of wild sheep, based on evidence that domestic sheep or goats can transfer pathogens to wild sheep. Literature (reviewed by Wehausen et al. 2011) and experimental evidence (Lawrence et al. 2010) support the goal that domestic sheep or goats should not concurrently occupy areas where conservation of wild sheep is a clearly stated management goal.

Effective separation does not necessarily require removal of domestic sheep or goats in all situations. However, the option of removing domestic sheep or goats should be included in an array of alternatives available to address this issue. In fact, some collaborative working groups (USAHA 2009) have recommended domestic goats not be allowed to graze in occupied bighorn sheep habitat because of their gregarious nature and tendency to wander. We are aware of the continuing debate and discussion (CAST 2008, USAHA 2009) between wildlife advocates and some domestic sheep or goat industry proponents and resource managers regarding the credibility or scientific merit of past findings; that debate is founded largely on criticisms of experimental design or rigor, and limitations of drawing inferences about natural disease events when compared to controlled experiments in confined settings. However, it is WAFWA's collective opinion that enough is known about potential pathogen transmission from domestic sheep or goats to wild sheep that efforts toward achieving effective separation are necessary and warranted.

Reducing risk of disease transmission on the landscape by minimizing or preventing association between wild and domestic sheep or goats is a key management strategy for WAFWA agencies (e.g., Colorado Division of Wildlife 2009, Montana Department of Fish, Wildlife and Parks 2009). Legislation in Utah (House Bill 240 Supplement, 2009), Wyoming (Senate Enrolled Act No. 30, 2009) and Idaho (Senate Bill 1232 amended, 2009) provides direction, authority and responsibilities for addressing feral or stray livestock that pose a disease transmission risk. Further, recent court rulings (e.g., U.S. District Court, Idaho Case 09-0507-BLW) have mandated separation between domestic sheep or goats and bighorn sheep, including mandatory non-use of grazing allotments where effective separation could not be assured.



Effective Separation

Principal federal land management agencies in the western U.S., BLM and USFS, continue to review, revise, and update policies on the management of domestic sheep or goats in wild sheep habitat (USDI BLM 1992, 1998, 2010; USDA Forest Service 2009). Additionally, several administrative units of the USFS (Northern Region, Rocky Mountain Region, Southwest Region, Intermountain Region, and the Pacific Southwest Region) have designated bighorn sheep as a “Sensitive Species,” thereby mandating special management emphasis. This includes: thorough reviews and analyses of management actions that could affect populations of bighorn sheep or their habitat to ensure their viability and to preclude demographic trends that would result in the need for Federal listing.

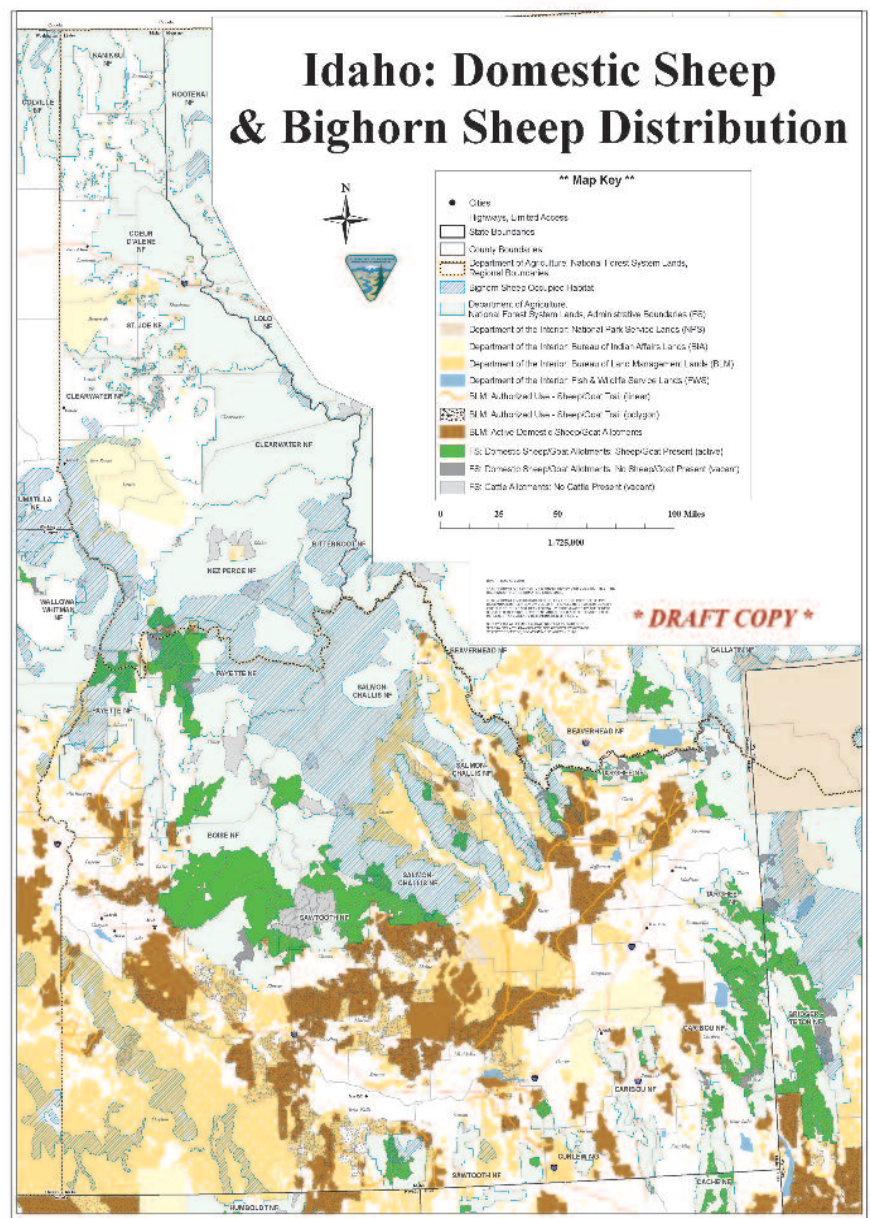
An interagency GIS-based decision-support tool and GIS coverage maps that overlay current bighorn sheep distribution with vacant and active domestic sheep or goat grazing allotments and trailing routes were finalized for 14 western states (WAFWA 2010a). These maps identify areas where association between domestic sheep or goats and bighorn sheep could occur on, or adjacent to, lands managed by BLM or USFS, and also identify areas that could provide spatial separation. The maps further provide a context for national policy development, and help identify situations where proactive management is necessary to minimize risk of association. Although risk of disease transmission from domestic sheep or goats to wild sheep is widely acknowledged by wildlife and land management agencies, a unified set of management guidelines for minimizing this risk has not yet been adopted.

In some cases, results of contact between domestic sheep or goats and wild sheep have been severe enough to endanger entire populations of the latter. In Idaho, legislation (Senate Bill 1232 amended, May 2009) mandated collaboration between the Idaho Department of Fish and Game and domestic sheep grazing permittees that identified BMPs to achieve effective separation between domestic sheep and wild sheep on both public and private lands. In specific situations, implementation of BMPs could lead to a reduced risk of association. In particular, BMPs implemented in open, gentle terrain where domestic sheep or goats can be easily controlled and monitored can reduce risk of association (Schommer 2009). Nevertheless, BMPs that work in one situation may not work in other situations (Schommer 2009).

Consequently, we recommend that managers take appropriate steps to minimize opportunities for association and, thereby, the potential for disease transmission in all situations.



Photo by: Bighorn Institute



Provided by: Chans O'Brien (USFS)

Management Recommendations

Management Recommendations

The recommendations that follow can be applied to state, provincial, and territorial wildlife agencies, federal/crown land management agencies, wild sheep conservation organizations, domestic sheep or goat producers or permittees, and private landowners, and have been strategically assigned to logical categories. It is imperative, however, that readers recognize these recommendations typically apply to multiple parties, and that they further recognize that a multi-disciplinary and collaborative approach will produce the best outcomes, both for wild sheep and for producers or permittees. Definitions of various terms used throughout this document are provided in Appendix A.

Although these recommendations have been developed by a working group largely comprised of wildlife agency personnel, cooperation between numerous concerned parties is critically important to deriving on-the-ground solutions (USAHA 2009, Wild Sheep Foundation 2011). Among these are state, provincial, and territorial wildlife agencies; federal/crown land management agencies; First Nation or tribal representatives; domestic sheep or goat producers or grazing permittees; agricultural industry representatives; wild sheep conservation organizations; environmental groups; academic institutions; and interested individuals. As a result of information contained herein, it is our hope that collaborative discussions will occur and that those discussions yield results in the form of innovative and collaborative site-specific delivery of programs such as the British Columbia Wild/Domestic Sheep Separation Program and the Wyoming Statewide Domestic Sheep/Bighorn Sheep Interaction Working Group.

Many anthropogenic and environmental factors (CAST 2008) influence the demographics and viability of wild sheep populations. Some factors affecting wild sheep population performance can be managed while others cannot. Nevertheless, the guiding principle of our effort has been “to seek effective separation” between wild sheep and domestic sheep or goats. There is no “one size fits all” risk assessment of respiratory disease transmission between wild sheep and domestic sheep or goats. However, a comprehensive risk assessment (qualitative and quantitative) is a critically important component for managing the potential for disease transmission.

We recommend that wild sheep managers design and implement management strategies by taking the first step of assessing and prioritizing conservation value and relative importance of wild sheep populations. The greater the conservation value and the greater the risk of association with domestic sheep or goats, the more aggressive and comprehensive a strategy to ensure effective separation should be. To ensure that is the case, we offer the following:



Photo by: Rebecca Barboza (CDFG)



Photo by: Larry Kruckenberg

Management Recommendations

RECOMMENDATIONS TO WAFWA AGENCIES

■ Historic and suitable but currently unoccupied wild sheep range should be identified, evaluated, and compared against currently-occupied wild sheep distribution and existing or potential areas where domestic sheep or goats may occur.

■ Risk assessments should be completed at least once per decade (more often if warranted) for existing and potential wild sheep habitat. These assessments should specifically identify where and to what extent wild sheep could interface with domestic sheep or goats, and the level of risk within those areas.

■ Following completion of site or herd-specific risk assessments, any translocations, population augmentations, or other restoration and management strategies for wild sheep should minimize the likelihood of association between wild sheep and domestic sheep or goats. Agencies should:

- Avoid translocations of wild sheep into areas with no reasonable likelihood of effective separation from domestic sheep or goats.
- Re-evaluate planned translocations of wild sheep to historical ranges as potential conflicts, landscape conditions, and habitat suitability change.
- Recognize that augmentation of a wild sheep herd from discrete source populations poses a risk of pathogen transfer (CAST 2008) and thus, only use source stock verified as healthy through a proper health assessment (WAFWA 2009) for translocations. Source herds should have extensive health histories and be regularly monitored to evaluate herd health. Wild sheep managers should evaluate tradeoffs between anticipated benefits such as demographic, behavioral and genetic interchange, and the potential consequences of mixing wild sheep from various source herds.
- Develop and employ mapping or modeling technology as well as ground based land use reviews prior to translocations to compare wild sheep distribution and movements with distribution of domestic sheep or goats. If a translocation is implemented and association with domestic sheep or goats occurs, or is likely to occur beyond an identified timeframe or pre-determined geographic area, domestic sheep or goat producers should be held harmless.

■ The higher the risk of association between wild sheep and domestic sheep or goats, the more intensively wild sheep herds should be monitored and managed. This is particularly important when considering “new” vs. “augmented” wild sheep populations.

- Site-specific protocols should be developed when association with domestic sheep or goats is probable. For example, decisions concerning percentage of translocated wild sheep that must be radio-collared



Photo by: Mike Cox (NDOW)



Photo by: Chase Fountain (TPWD)

Management Recommendations

for achieving desired monitoring intensities should in part, be based upon the subsequent level of risk of association with domestic sheep or goats.

- Intensive monitoring provides a mechanism for determining proximity of wild sheep to domestic sheep or goats and for evaluating post-release habitat use and movements.
- Budgets for wild sheep translocation projects should include adequate funding for long-term monitoring.

■ Wild sheep managers should identify, analyze, and evaluate the implications of connectivity and movement corridors between largely insular herds comprising a meta-population against opportunities for increased association with domestic sheep or goats. Analyses should include distribution and continuity (Mack 2008) among populations of wild sheep and the anticipated frequency of movement among or within wild sheep range. In doing so, the benefits

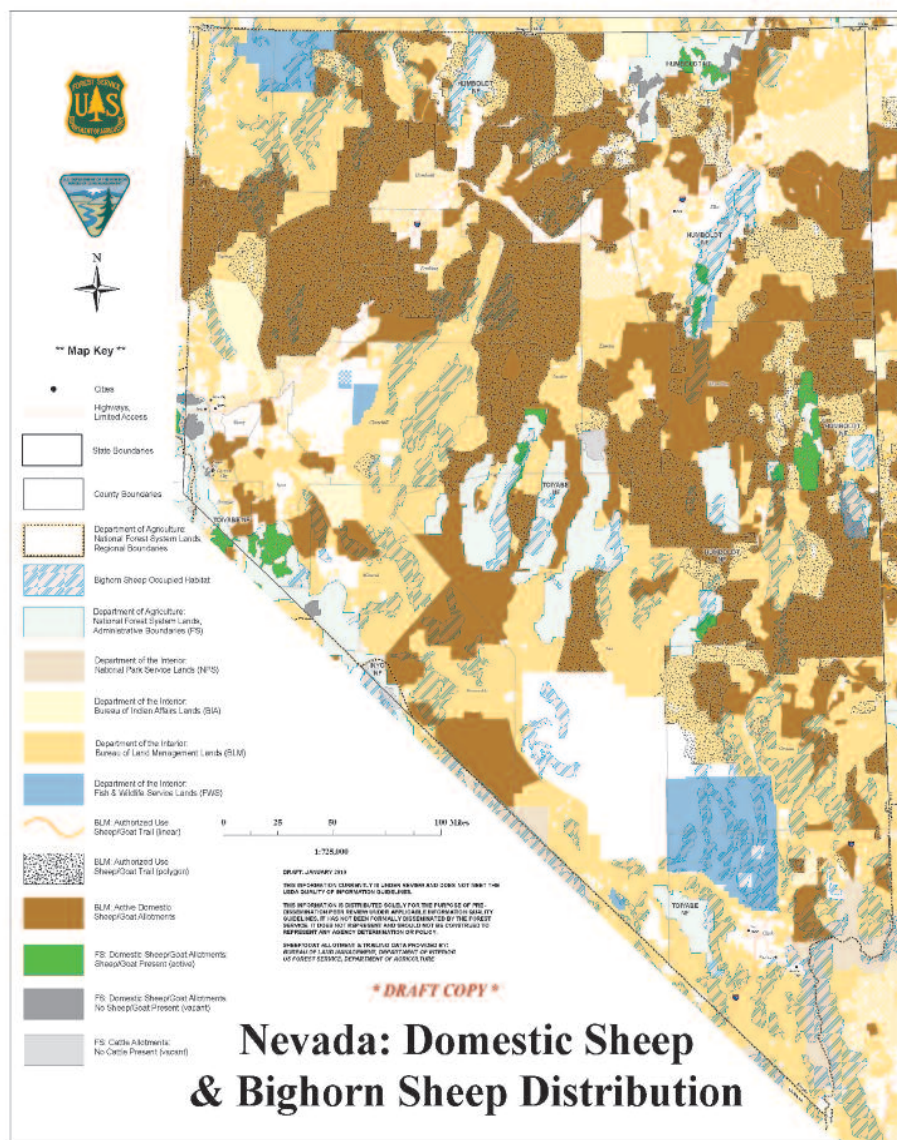
of genetic interchange and its resultant implications for population viability, must be weighed against the risks of disease transmission (Bleich et al. 1990), especially if dispersing or wandering wild sheep could travel across domestic sheep or goat grazing allotments or trailing routes, private land holdings or other areas where the potential transfer of endemic pathogens from an infected wild herd to a naïve herd could occur.

■ Removal of wild sheep known, or suspected to have closely associated with domestic sheep or goats is considered to be an effective management tool. Atypical movements by wild sheep can heighten risk of association with domestic sheep or goats. Additional measures to achieve effective separation should be implemented if such association occurs. However, removal of wild sheep from occupied, normally-anticipated wild sheep range is not always the best management option.

Continuous risk of association exists during active grazing seasons when domestic sheep or goats are grazed within normally-anticipated wild sheep range. Thus, removal of individual wild sheep is an ineffective method for maintaining separation, and has potentially negative consequences for population viability. Removal of wild sheep should occur only after critical evaluation and further implementation of measures designed to minimize association and enhance effective separation.

■ Wild sheep populations should have pre-determined population objectives, and should be managed at agreed-upon densities to minimize the potential for dispersal. Because some dispersal occurs regardless of population density, some risk of association is always present if domestic sheep or goats are within range of dispersing wild sheep.

■ Agencies should develop a written protocol to be implemented when association between wild sheep and domestic sheep or goats is confirmed. Notification requirements, appropriate response and post-contact monitoring options for both domestic sheep and goats and dispersing or wandering wild sheep should be included. Moreover, wildlife agencies should collaborate with agricultural agencies, land management agencies, producers and permittees, grazing industry representatives,



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and wild sheep advocates to develop an effective, efficient, and legal protocol to be implemented when feral or abandoned domestic sheep or goats threaten to associate with wild sheep but for which no owner can be identified. Written protocol examples are provided in Appendix B (British Columbia Fish, Wildlife and Habitat Management Branch) and Appendix C (Wyoming Game and Fish Department).

- Wildlife agencies should develop databases as a system to report, record, and summarize association between wild sheep and domestic sheep or goats and its outcome; the WAFWA WSWG website (<http://www.wafwa.org/html/wswg.shtml>) would be a logical host. Further, wildlife managers and federal/crown land managers should encourage prompt reporting by the public of observed proximity between wild sheep and domestic sheep or goats.

- Wild sheep managers should coordinate with local weed or pest management districts, or other applicable agencies or organizations involved with weed or vegetation management, to preclude the use of domestic sheep or goats for noxious weed or vegetation control in areas where association with wild sheep is likely to occur. Agencies should provide educational information and offer assistance to such districts regarding disease risks associated with domestic sheep or goats. Specific guidelines (Pybus et al. 1994) have already been developed and implemented in British Columbia, and are available at: <http://www.for.gov.bc.ca/hfp/publications/00006/>.

- Specific protocols for sampling, testing prior to translocation, and responding to disease outbreaks should be developed and standardized to the extent practical across state and federal jurisdictions. Several capture and disease-testing protocols have been developed and are available to wild sheep managers (Foster 2004, UC-Davis 2007, WAFWA 2009). Protocols should be reviewed and updated as necessary by the WAFWA Wildlife Health Committee (WHC) and presented to WAFWA Directors for endorsement. Once endorsed, agencies should implement the protocols, and the WHC should lead an effort to further refine and ensure implementation of said protocols.

- Agencies should coordinate and pool resources to support the ongoing laboratory detection and interpretation of important diseases of wild sheep. Furthermore, wild sheep managers should support data sharing and development and use of standardized protocols (WAFWA 2009). Interagency communication between wildlife disease experts such as the WAFWA Wildlife Health Committee (WHC) should be encouraged to enhance strategies for monitoring, managing and improving health of wild sheep populations through cooperative efforts.

- Wild sheep management agencies should develop educational materials and outreach programs to identify and interpret the risk of association between wild sheep and domestic sheep or goats for producer groups, owners of small and large farm flocks, animals used for packing and 4-H animals. In some cases, regulation may be necessary to maintain separation.

RECOMMENDATIONS TO BLM, USFS, PARKS, PROTECTED AREAS AND OTHER APPLICABLE LAND MANAGEMENT AGENCIES

- Joint federal land management agency guidelines on management of domestic sheep or goats in wild sheep habitat should be developed and included in broad agency policy documents. Guidelines should be based on the need to minimize risk of association and provide effective separation between domestic sheep or goats and wild sheep.



Photo by: Todd Nordeen (NGPC)



Photo by: Debra Hamilton (CDFG)

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■ The use of domestic sheep or goats as pack animals by persons that travel in identified wild sheep habitat should be prohibited by the appropriate management agency (e.g., USDA Forest Service 2011). Where legislation or regulations are not already in place, an outreach program to inform



Photo by: Ted Borda (Borda Land & Sheep Company)

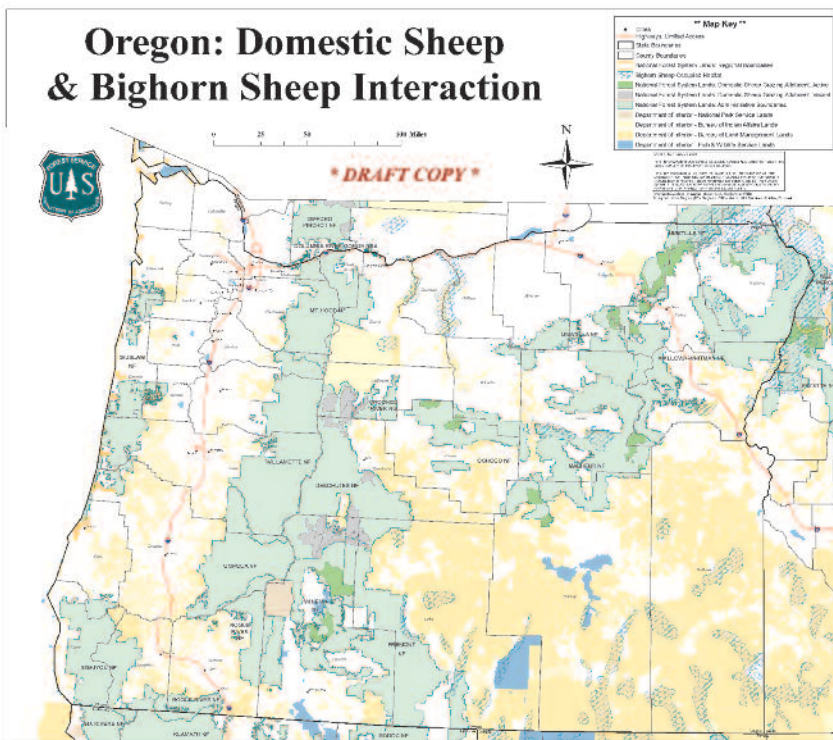
- Land management agencies that regulate or are responsible for domestic sheep or goat grazing allotments, trailing routes, vegetation management, use as pack stock, or any other uses involving domestic sheep or goats should only authorize such use(s) outside of occupied wild sheep range.

- Land management agencies should map active and inactive domestic sheep or goat grazing allotments and trailing routes, including information on dates of use and contact information for responsible grazing or trailing permittees.

■ Land management agencies must ensure that advance written instructions (such as USFS Annual Operating Instructions) exist, and that they address management, retrieval, and disposition of domestic sheep or goats present on public lands prior to or after permitted grazing or trailing dates.

- Land management agencies should work collaboratively with state, provincial, and territorial wildlife and agricultural interests to develop written agreements that address management, retrieval, and disposition of domestic sheep or goats occupying public lands where there is no permitted use. Such agreements should also address the presence of feral sheep or goats and other exotic ungulates, especially ovines such as aoudad, red sheep, urial, or argali that are detected on public lands.

- Land management agencies should review domestic sheep allotment boundaries or other use areas, such as trailing routes, and reconfigure boundaries or routes to avoid or minimize overlap with occupied wild sheep habitat. Techniques available to accomplish this include the use of geographic or topographic



Provided by: Chans O'Brien (USFS)



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barriers that enhance species separation, and temporal or spatial separation resulting from implementation of novel domestic sheep or goat grazing management strategies.

- Land management agencies should undertake habitat enhancements that improve wild sheep habitat outside allotment boundaries in an effort to attract wild sheep away from domestic sheep allotments.

- Land management agencies should undertake water developments to divert wild sheep away from domestic sheep allotments or domestic sheep or goats away from areas used by wild sheep.

- Land management agencies should ensure that Annual Operating Instructions require careful management and vigilant herding to minimize potential association between wild sheep and stray domestic sheep or goats. A count-on, count-off inventory of domestic sheep or goats must be required as a condition of operation with follow-up provisions to account for missing livestock.

- In areas of high risk of association, trucking should be required to minimize risks associated with trailing. Trucking of domestic sheep or goats is preferred to trailing because there is less chance of straying and, thereby, less likelihood of association with wild sheep, particularly when domestic sheep are in estrus.

- Land management agencies should require marking of all permitted domestic sheep and goats to provide for rapid ownership identification of stray animals.

- In the event of trailing, on-site compliance monitoring to minimize strays must be conducted by the permittee or the land management agency.

- Land use or resource management plans should explicitly address the potential for domestic sheep or goats to associate with wild sheep. Land use plans should evaluate the suitability of permitting activities involving domestic sheep or goats, and determine the best course of action with respect to wild sheep conservation. Plans should also identify general areas of public land where domestic sheep or goats cannot be permitted for weed control, commercial grazing, recreational packing, vegetation management, or other uses.

- Land management agencies should coordinate with appropriate entities involved in weed control programs that use domestic sheep or goats on public or Crown lands (Pybus et al. 1994), adjoining private lands, or state, provincial, and territorial wildlife habitat management areas to minimize risk of association between domestic sheep or goats and wild sheep.

- Within occupied or suitable wild sheep habitat, where topography, vegetation, and other parameters allow, conversions of allotments from domestic sheep or goats to types of domestic livestock that pose a lower risk of disease transmission to wild sheep should be implemented.

- Within suitable, historic wild sheep habitat not currently occupied by wild sheep, agencies should not convert cattle grazing allotments to domestic sheep or goat grazing, or allow trailing if restoration of wild sheep populations is an agency goal.



Photo by: AZGFD



Photo by: Justin Binfet (WGFD)

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■ Under emergency conditions, stocking of allotments not currently under permit to domestic sheep or goats should be permitted only after an adequate risk assessment has been completed. Any such assessment must include appropriate documentation and the conclusion that effective separation can be assured, and can be accomplished via project-level NEPA analysis.

■ Land management agencies should incorporate state, provincial, or territorial wild sheep management plans either in, or as supplements to, federal resource or land use management plans, and collaborate with wildlife agencies to ensure comprehensive risk assessments (Clifford et al. 2009, USDA Forest Service 2010a, b) of domestic sheep or goat grazing allotments or trailing routes in wild sheep habitat are thorough and complete. To accomplish this objective, training adequate to allow the preparation of such assessments must be provided.

Photo by: Mike Cox (NDOW)



■ Where mandatory buffer zones (frequently cited as a minimum of 9 airline miles [14.5 km]) between domestic sheep or goats and wild sheep have been used to minimize association, it should be recognized that buffer zones apply to herds or populations of wild sheep, rather than individual wandering wild sheep. In some cases, buffer zones have been effective in reducing association between wild sheep and domestic sheep or goats. However, in contiguous wild sheep habitat where movements by wild sheep have the potential to exceed *a priori* expectations, buffer zones may not be effective or practical (Schommer and Woolever 2001).

■ Topographic features or other natural or man-made barriers (e.g., fenced, interstate highways) can be effective in minimizing association between wild sheep and domestic sheep or goats. Site-specific risk assessments should be completed to evaluate the efficacy of using natural barriers, defined buffer zones, or other actions to minimize risk of contact. Given the wide range of circumstances that exists across jurisdictions, buffer zones may not be needed in all situations. Conversely, buffer zones should not be precluded as an effective method to address potential association between wild sheep and domestic sheep or goats.

■ Land management agencies, in collaboration with jurisdictional domestic sheep or goat health agencies, should work with producers and permittees to prevent turnout or use of sick or diseased domestic sheep or goats on grazing allotments and trailing routes. Sick or diseased domestic sheep or goats can increase risk of association with wild sheep because they likely are less able to keep up with their bands and are more prone to straying. Sick or diseased animals observed on the range should be reported to land management agency personnel immediately, and inter-agency coordination to address the situation should promptly occur. Further, responsible agencies must require that domestic sheep or goats are in good health before being turned out. For example, Alberta and British Columbia have developed health certification protocols (Pybus et al. 1994) that must be complied with before domestic sheep are turned out for vegetation management in conifer regeneration efforts (available at: <http://www.for.gov.bc.ca/hfp/publications/00006/>). We emphasize that the higher the risk of association between domestic sheep or goats with wild sheep, the higher the certainty of domestic animal health should be. Further, it must be recognized that even clinically healthy domestic sheep or goats can still carry pathogens that are transmissible to wild sheep, and thus, pose a significant risk to wild sheep.

Photo by: Todd Nordeen (NGPC)



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■ Proportional to risk of association between domestic sheep or goats and wild sheep, land management agencies should work with stakeholders to implement a variety of management practices. Examples include: herders, dogs or other guarding animals trained to repel animals foreign to domestic sheep bands or goat flocks (wandering wild sheep or various predators), regular counts, removal of sick animals, confinement of domestic sheep or goats at night, adequate fencing configurations, covenants, allotment retirements, conversion of class of livestock, trucking versus trailing, and others. Effectiveness of management practices designed to reduce risk of association are not proven (Baumer et al. 2009, Schommer 2009) and therefore should not be solely relied upon to achieve effective separation. Such practices could however, help achieve separation when applied outside of occupied wild sheep range or connected and potentially mitigate impacts associated with straying domestic sheep or goats, or wandering wild sheep.

■ Land management agencies and wildlife agencies should cooperatively manage for quality wild sheep habitat and routinely monitor habitat to detect changes in condition.

■ In areas where association between wild sheep and domestic sheep or goats is likely, land management agencies should post advisory signs at trailheads, campgrounds, and other high-use areas that are designed to educate visitors about the issue of interaction and to encourage prompt reporting of association of wild sheep with domestic sheep or goats. Agencies should also ensure that individuals keep dogs under immediate voice control or on leash to prevent scattering of domestic sheep or goats in permitted areas, or disturbances to wild sheep.

■ Land management agencies should clearly define the processes, protocols, and timelines for short-term or emergency management actions when intervention is needed to minimize risk of association between wild sheep and domestic sheep or goats.

■ Land management agencies should develop programs to foster and recognize the benefits of compliance, cooperation, and cost-sharing in efforts to prevent commingling of wild sheep and domestic sheep or goats on shared ranges.

■ In collaboration with wild sheep management agencies, land management agencies should investigate and implement an option to allow the permittee or producer, or appropriate agency representatives, to remove commingling wild sheep and, where not already established, develop or clarify legal authority for removing stray domestic sheep from public lands by lethal means.

■ Risk assessment should be conducted on an appropriate geographic scale regardless of jurisdictional boundaries. Recognizing the limits of regulatory authority, land management agencies should consider private in-holdings and adjacent private lands when conducting risk assessments.

■ Land management agencies should closely evaluate timing of permitted domestic sheep or goat grazing or trailing activities to reduce risk of disease transmission. For example, grazing estrous domestic females heightens



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attraction and increases the probability of association between wild sheep and domestic sheep, and should be eliminated where benefits can be accrued.

■ In areas of high risk of association between wild sheep and domestic sheep or goats, agencies and permittees should ensure enhanced monitoring of grazing and trailing patterns using global positioning system (GPS) collars or other technology that provide detailed data on movements and grazing patterns. While enhanced monitoring will not reduce risk of association, it is vital for development of meaningful risk assessments and to ensure appropriate management recommendations are taken to achieve effective separation.



Photo by: Mike Gox (NDOW)



Photo by: Helen Schwantje (BC FLNRO)

RECOMMENDATIONS TO WILD SHEEP AND OTHER CONSERVATION ORGANIZATIONS

- Recognize and support efforts of wild sheep management agencies and industry leaders in maintaining effective separation.
- Assist wildlife and land management agencies with development of informational brochures and other materials that identify and explain risk of association between wild sheep and domestic sheep or goats.
- Assist wildlife and land management agencies with educational efforts regarding risks associated with the use of domestic sheep or goats as pack animals in wild sheep habitat. If use is authorized, encourage participants to closely control, tether, and night-pen their pack stock. Encourage prompt reporting of association between wild sheep and domestic sheep or goats, and promote a reporting system for monitoring association between wild sheep and domestic sheep or goats.
- Maintain or establish open lines of communication with domestic sheep or goat producers and industry organizations to reduce polarization. Jointly organized and cooperatively-funded workshops on risk assessment, identification of practical strategies to achieve effective separation, development and distribution of pamphlets or brochures, and public speaking opportunities are tangible examples of collaborative, multi-disciplinary approaches to address potential disease transmission.
- Continue to negotiate alternatives or incentives for domestic sheep or goat permittees to shift their operations to grazing allotments outside of wild sheep habitat. Advocate that permittees convert to a different class of livestock with lower risk of disease transmission or waive permitted domestic sheep or goat use in areas where risk assessment indicates high potential for association with wild sheep.

- Encourage and support development and funding of cooperative research, and encourage agencies and conservation groups to commit resources necessary to maintain wild sheep populations.

SUGGESTED MANAGEMENT PRACTICES FOR DOMESTIC SHEEP AND GOAT PERMITTEES

The following suggestions are based largely on recommendations provided by CAST (2008), Baumer et al. (2009), or USAHA (2009), and are intended to provide a responsible and common-sense approach for reducing risk of association. However, there is no science-based evidence or evaluation that assesses the effectiveness of these actions to reduce risk or enhance separation (Schommer 2009).



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■ Implement the following reporting and record keeping procedures or use an existing standard such as the BC (Appendix B) or Wyoming (Appendix C) models:

- Require prompt, accurate reporting by herders working on domestic sheep or goat grazing allotments where association of wild sheep with domestic sheep or goats is possible.
- Support fluency in English or translators for foreign herders in order to facilitate accurate reporting.
- Require sheepherders to use cellular or satellite phones or two-way radios, and location equipment such as GPS receivers to report and record grazing movements and encounters with wild sheep. Seek cost-sharing partnerships for providing communications equipment when an operator changes grazing management practices for the sole purpose of minimizing domestic sheep association with wild sheep. Partnerships could include wildlife management agencies, federal land managers, or private organizations.
- Require herders to record GPS locations, counts, losses and other information in a log book.

■ Place only experienced, informed and responsible sheepherders on allotments located near wild sheep habitat.

■ Ensure that all domestics are individually marked and traceable to source flocks.

■ Conduct full counts when trailing, immediately any time scattering occurs and regularly during general grazing.

■ Develop agreements between permittees and wildlife agencies that provide for locating and reacquiring all stray domestic sheep, either dead or alive. In the event of missing domestic sheep, a comprehensive search should be initiated immediately and the land manager and state wildlife agency must be notified of missing and subsequent recovery of animals.

■ Develop a detection and response protocol that includes:

- Reporting of wild sheep and domestic sheep associations (animal counts and GPS location) to the appropriate wildlife agency.
- Reporting of stray or missing domestic sheep to the land management agency who will, in turn, report that information to the wildlife agency.
- Removal of stray domestic sheep by the permittee, land manager or wildlife agency personnel.
- Removal of individual commingling wild sheep by wildlife agency personnel.
- Collection of standardized diagnostic samples from stray domestic sheep or commingling wild sheep.

■ Utilize the following trailing procedures:

- Conduct full counts when moving on and off each allotment/grazing site.

- Truck domestic sheep through “driveway” areas that pass through occupied wild sheep habitat.
- Truck in water (if needed) to reduce straying.
- Immediately remove animals unable to stay with the flock/herd and move them to a base property.
- Avoid trailing more than 5 miles per day and stop trailing when sheep or lambs show signs of fatigue. Provide for a “babysitter” or removal of lagging sheep when trailing.
- In the event that all animals cannot be accounted for, the permittee must advise the responsible agency and initiate efforts to locate missing animals and implement removal protocol as necessary.

■ Sick domestic sheep should be removed from allotments immediately and must never be abandoned.





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- Select herder's camp, nighttime bedding ground, and midday bedding ground locations that maintain communication between guard dogs and herding dogs by smell, sound (barking) and sight, and to take advantage of differences in the sleep cycles of guard dog and herding dogs. Place mature and effective guard dogs and herding dogs with domestic sheep (at least 2 of each per 1000 animals) and do not use female dogs in heat.

- If grazing on federal lands, comply with established "bed ground" standards. Where conditions permit, construct temporary electric or boundary fences to ensure that domestic sheep remain within selected bedding grounds.

SUGGESTED MANAGEMENT PRACTICES ON PRIVATE LANDS

- Recognize that domestic sheep or goat farming on private lands can influence wild sheep population viability on adjacent public or other private lands.

- Report any observed association between wild sheep and domestic sheep or goats on or near private land to the appropriate wildlife conservation agency.

- Cooperate with wildlife agencies in reporting and removing feral sheep or goats and other exotic bovine ungulates such as aoudad, red sheep, urial, or argali that are detected within or near wild sheep habitat.

- Participate in cooperative educational efforts to enhance understanding of the issues of disease transmission between domestic sheep or goats and wild sheep.

- Do not release or leave unattended domestic sheep or goats in areas where they may seek, or be sought, by wild sheep.

- Cooperate with appropriate agencies, agricultural and producer associations, conservation organizations, and other interested stakeholders to develop effective, comprehensive risk management approaches to help ensure effective separation between wild sheep and domestic sheep or goats, consistent with private property rights in and near wild sheep habitat.

- Possible approaches include, but are not limited to, changing species or class of livestock, purchase of land or the domestic sheep or goats, use of methods to ensure physical separation, or development of conservation incentives, bylaws, covenants, or legislation.

- Consider partnerships with non-governmental organizations and wild sheep advocate groups for cost sharing on risk management/mitigation strategies such

as fencing, or other domestic sheep or goat management actions that reduce risk of disease transmission from private flocks to wild sheep.

- Support "effective separation" fencing standards that are designed to prevent nose-to-nose contact and aerosol transmission through adequate physical distance, in order to reduce transmission of respiratory disease agents. Examples include: electric outrigger fences (2 feet from page (woven) wire fencing) and double fencing (two page-wire fences with a minimum spacing of at least 10 feet). A combination of fencing methods with or without the use of effective livestock guardian dogs may be most effective to ensure that wild sheep do not physically contact domestic sheep or goats on private land.

- Participate in or support cooperative research to enhance understanding and test mitigation protocols for disease risk management.

- Carefully consider the consequences of using domestic sheep or goats for weed control on private lands where association with wild sheep could occur. Work with agencies to develop alternative weed management strategies to reduce risk of association, while adequately managing weed problems.

Photo by: David Wetzel (Texas Bighorn Society)



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Glossary of Terms

Allotment: A portion of a landscape where livestock grazing of a plant community is prescribed according to a specific land use plan or legally defined regulatory authority.

Annual Operating Instructions: Specific language included in a term grazing or trailing permit file; reviewed each year with the permittee, prior to turnout of livestock on a grazing allotment or trailing route.

Association: Close proximity between wild sheep and domestic sheep or goats, potentially leading to direct physical contact and potential disease transmission.

Augment: To intentionally introduce wild sheep from one or more source populations into another existing wild sheep population, to enhance the recipient population demographically or genetically.

Buffer zone: A defined and delineated space on a landscape established by wildlife managers to reduce association and the potential for disease transmission between wild and domestic sheep or goats across that geographic space.

Bighorn sheep: A member of the species *Ovis canadensis* found throughout the mountains of western North America from the Peace River in Canada to northern Mexico and east to the Badlands of the Dakotas.

Contact: Direct contact between body parts of two animals during which a disease might be transmitted from one to another. In this document, “contact” typically refers to nose-to-nose or face-to-face interactions that may lead to the transmission of respiratory disease via secretions or aerosols. Synonymous with “Interaction.”

Connectivity: Creating or maintaining networks of habitat that connect fragmented habitats, thus linking population segments of wildlife. Connectivity allows gene flow and enhances long-term species survival.

Conservation Incentives: In direct contrast to regulation-based conservation, incentive-based conservation provides economic, management or esthetic benefits to individuals or corporations to encourage them to conduct management activities that have positive conservation consequence to wildlife or wildlife habitat. Examples are: private land conservation easements, direct lease agreements for grazing rights for conservation purposes, or a trade/exchange of equal value grazing rights among various partners to minimize wildlife-domestic livestock conflict.

Die-off: A large-scale mortality event that impacts many animals from a population and may have significant demographic consequence for the long-term persistence of that population. In this report, such mortality events are usually caused by respiratory disease epidemics involving bacterial or other pathogens alone or in various combinations.

Disease: The word disease means literally “free of ease.” Disease is any impairment that modifies or interferes with normal functions of an animal, including responses to environmental factors such as nutrition, toxicants, and climate. Typically, disease involves transmission of, and exposure to, some infectious agent but it may involve non-infectious causes such as congenital defects.

Dispersal: The process whereby individuals leave one habitat or landscape to seek another habitat or landscape in which to live.

Double fencing: Two fences running parallel around a landscape or pasture to prevent contact between animals across the fence line, designed to inhibit disease transmission.

Effective separation: Spatial or temporal separation between wild sheep and domestic sheep or goats, resulting in minimal risk of contact and subsequent transmission of respiratory disease between animal groups.

Feral: An animal of a domestic species that resides in a non-domestic setting and is not presently owned or controlled.

Historic habitat: Based on historic records, landscape that was previously occupied by bighorn sheep and thought to have provided necessary requirements to sustain a wild sheep population through time.

Interaction: Direct contact between body parts of two animals during which a pathogen might be transmitted from one to another. In this document, “interaction” typically refers to nose-to-nose or face-to-face interaction that may lead to the transmission of respiratory disease via secretions or aerosols. Synonymous with “Contact”.

Metapopulation: An assemblage of populations, or a system of local populations (demes) connected by movement of individuals (dispersal) among various population segments.

Movement corridor: Routes that facilitate movement of animals between habitat fragments.



Appendix A

Occupied habitat/range: Suitable habitat in which a wild sheep population currently exists.

Preferred: A specific management action that *should* be chosen over another, whenever possible:

Radio collars: Transmitters fitted on neckband material to monitor animal locations.

Global Positioning System (GPS): A radio transmitter fitted on neckband material linked with orbiting satellites; animal locations can be precisely triangulated from space, with the location data then electronically stored in a memory chip or transmitted by various methods for data retrieval.

Very High Frequency (VHF): A radio transmitter fitted to neckband material transmitting in the Very High Frequency range that can be located from the ground or aircraft using a telemetry receiver.

Removal: Physical extraction of domestic sheep or goats, or wild sheep, to eliminate (permanently or temporarily) occupancy of that range or habitat.

Risk/Risk Assessment/Risk Management: In this context, evaluation of the probability that a wild sheep population could experience a disease event with subsequent demographic impacts. Identification of what factors might contribute to the probability of a disease event. Management actions taken to reduce the probability of exposure and/or infection among or between animals. Examples of risk management include separation of infected and non-infected animals, treatment of infected individuals, vaccination, manipulations of the host environment, or manipulations of the host population.

- Qualitative Risk Assessment: Interpretation and analysis of factors that cannot necessarily be measured.
- Quantitative Risk Assessment: Use of tangible data and measurements.

Spatial separation: A defined physical distance between animal populations.

Stray: A domestic sheep or goat physically separated from its flock or band.

Stressor: A specific action or condition that causes an animal to experience stress and the subsequent physiological results of that stress.

Suitable habitat: Landscape that has all necessary habitat requirements to sustain a wild sheep population through time.

Temporal separation: Segregating animal populations over time to prevent association, such that they may occupy the same physical space but at different times.

Thinhorn sheep: A member of the species *Ovis dalli* occurring in Alaska, Yukon Territory, Northwest Territories, and northern British Columbia.

Transmission: The physical transfer (direct or indirect mechanisms) of a disease agent from one animal to another, either within an animal population or between animal populations. In some instances, transmission can lead to full expression of disease in individuals or populations.

Transplant: An intentional movement of wild sheep from a source population to other suitable wild sheep habitat, either currently occupied or not. (Also called “translocation” in some documents.)

Trailing: The planned ambulatory movement of domestic sheep or goats across a landscape or within a corridor to reach a destination where grazing or use will be allowed.

Unoccupied habitat/range: Suitable habitat in which a wild sheep population does not currently exist.

Viability: The demographic and genetic status of an animal population whereby long-term persistence is likely.

Wandering Wild Sheep: Wild sheep, primarily but not always young, sexually-mature rams, occasionally traveling outside of normally anticipated or expected wild sheep range and adjacent habitat. Removal of wandering wild sheep typically does not have population-level implications for wild sheep. Conversely, failure to respond to wandering wild sheep may result in significant, adverse population-level impacts.

British Columbia Domestic-Wild Sheep Separation Project Contact Protocol

The following protocols outline *the steps to be taken when reports of wild sheep contact with domestic sheep are received by the Ministry of Environment* in one of several ways:

1. Regular report from public to regional office (Conservation Officer Service or Wildlife Section):

- Contact reported to Regional office.
- Assessment of situation by sheep biologist and COS, in consultation with wildlife veterinarian
- If close contact is confirmed and is considered a high risk situation, consider the following options:
 - a. Kill bighorn and save carcass – sample bighorn and/or domestics in consultation with wildlife veterinarian
 - b. Continue to monitor bighorn herd in area – observe and record general signs of health
 - c. Do nothing – but keep records
- If contact is unsubstantiated/considered low risk, continue to monitor bighorn herd in area, alert and encourage mitigation measures with domestic producers in area to ensure separation.

2. Regular report from public to Call Line.

- Contact reported to Call Line; Call Line staff forwards to regional COS.
- Assessment of situation by COS and sheep biologist, in consultation with wildlife veterinarian
- If close contact is confirmed and is considered a high risk situation, consider the following options:
 - a. Kill bighorn and save carcass – sample bighorn and/or domestics in consultation with wildlife veterinarian
 - b. Continue to monitor bighorn herd in area – observe and record general signs of health
 - c. Do nothing – but keep records
- If contact is unsubstantiated/considered low risk, continue to monitor bighorn herd in area, alert and encourage mitigation measures with domestic producers in area to ensure separation.

3. Out of hours call from public to Call Line.

- Contact reported to Call Line; Call Line staff forwards to regional COS officer-on-call.
- Assessment of situation by COS officer-on-call – contacts sheep biologist and wildlife veterinarian, if possible for consultation
- If sheep biologist and wildlife veterinarian cannot be contacted, biologist and veterinarian will support COS decision and action. COS will inform sheep biologist and wildlife veterinarian by email of the situation and action taken.
- If close contact is confirmed and is considered a high risk situation, consider the following options:
 - a. Kill bighorn and save carcass – sample bighorn and/or domestics in consultation with wildlife veterinarian
 - b. Continue to monitor bighorn herd in area – observe and record general signs of health
 - c. Do nothing – but keep records
- If contact is unsubstantiated/considered low risk, continue to monitor bighorn herd in area, alert and encourage mitigation measures with domestic producers in area to ensure separation.

Appendix C



WYOMING GAME AND FISH DEPARTMENT

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MEMORANDUM

TO: Wildlife Division Employees

FROM: Jay Lawson, Chief, Wildlife Division

COPY TO: Terry Cleveland, Gregg Arthur, File

SUBJECT: **PROTOCOL FOR HANDLING THE COMMINGLING
OF BIGHORN SHEEP AND DOMESTIC SHEEP/GOATS**

Due to the threat of disease transmission and subsequent bighorn sheep die-offs, the following protocol should be followed.

Wandering Bighorn Sheep:

Where there is known, suspected, or likely contact by a wandering bighorn sheep with domestic sheep/goats:

- If possible, that bighorn(s) should be live-captured and transported (one-way) to our Sybille Research Unit.
- If that bighorn(s) cannot be live-captured, that bighorn(s) should be lethally removed (per authority of Chapter 56) and, if possible, transported (either whole or samples) to our Sybille Unit or our WGFD Lab in Laramie.

Stray Domestic Sheep/Goat:

Where there is known, suspected, or likely contact by a stray domestic sheep/goat with bighorn sheep:

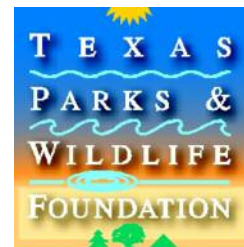
- The owner of such livestock should be notified and asked to remove the stray sheep/goat to eliminate the threat of disease transmission; however, it will be the owner's prerogative to determine what course of action should be taken.

Reporting:

All documented commingling and any actions taken must be reported to the employee's immediate supervisor, Wildlife Administration as well as the Bighorn Sheep Working Group Chairman, presently Kevin Hurley.

"Conserving Wildlife - Serving People"

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