Managing domestic and wild caprinae to limit pathogen transmission risk

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Executive summary

Limiting the burden that *Mycoplasma ovipneumoniae* (M. ovi) places on wildlife and livestock health is an important consideration for conservationists and livestock producers. Past exposure to M. ovi can be determined through a serological ("cELISA") test, while current active infection is determined through a PCR-based test. M. ovi can be a major threat to wild sheep health, and although its burden on domestic sheep tends to be more limited, it is associated with lower lamb weaning weights and diminished flock health.

Currently, there is no effective vaccine against M. ovi, and none is likely to emerge in the immediate future. Several antibiotic treatments have been explored, and some appear to be effective. These treatments show early promise in several cases, however they can be costly, and should be accompanied by careful biosecurity planning and consultation with a veterinarian.

M. ovi has been detected in other host species besides sheep and goats, in particular muskox and caribou. While detections have been reported in moose, cattle, and several other species, these detections are not consistently replicated across labs, and more work is needed to document whether and how frequently M. ovi is present in those species. Most of those hosts have limited range overlap with wild sheep, and therefore pose a lower risk of wild sheep populations than domestic sheep and goats. Maintaining separation between wild sheep and domestic sheep goats is still the best method for protecting wild sheep herds from infection and diseases. In particular, Crown Land grazing (tenures or permits) of domestic sheep and goats within wild sheep habitats should be avoided.

One strategy for maintaining separation is constructing and maintaining good fence. Wild sheep are excellent jumpers, so high-quality fencing is often required to retain separation. However, combining electric fencing with other fence types has shown promise in an early trial, and may be a good lower-cost option.

Relatively little is known about M. ovi in mountain goats. We know that it can cause disease, with losses similar to those incurred on bighorn sheep in some cases, but the frequency of these occurrences and the range of outcomes mountain goat herds might experience remain a topic of on-going investigation.

I. Introductory note

Respiratory disease places a major burden on the well-being and growth of bighorn sheep populations throughout western North America (Cassirer & Sinclair 2007; Cassirer et al. 2017). Current research indicates that despite the diversity of lethal bacteria implicated in bighorn pneumonia mortalities, a single agent, *Mycoplasma ovipneuoniae*, often facilitates these infections by impeding ciliary functionality in the upper respiratory tract (Besser et al. 2008; Dassanayake et al. 2010). *M. ovipneumoniae* is carried at high prevalence (Manlove et al. 2019) and diversity (Kamath et al. 2019) in the upper respiratory tracts of domestic sheep, but while it does place some constraints on domestic sheep health (Besser et al. 2019; Manlove et al. 2019), these costs pale in comparison to the effect *M. ovipneumoniae* has on wild sheep. As such, wildlife management agencies throughout western North America have prioritized separation of bighorn and domestic sheep, and many agencies are engaging in efforts to reduce the prevalence of *M. ovipneumoniae* from domestic and bighorn flocks.

This document was prepared in response to a request from British Columbia to assess management actions and provide advice on limiting disease transmission risk between domestic caprinae and wild sheep and goats. While I was primarily responsible for assembly of the document (and am responsible for any errors or oversights it contains), its contents are drawn from the peer reviewed literature; state, provincial, and federal reports from the United States and Canada; and the lived experience of a wide range of experts on *M. ovipneumoniae* in both bighorn and domestic sheep. The collective experience of this expert group, and their willingness to engage intellectually on strategies for *M. ovipneumoniae* management, dramatically improved the quality of this endeavor. I have tried to credit particular individuals without whom the contents would be inextricably altered in the Acknowledgements portion of each section, and I anticipate additional input in the final iteration of this document.

Finally, **research into *Mycoplasma ovipneumoniae* and its consequences on wildlife and livestock hosts is moving rapidly, thus I anticipate that some elements of this report (particularly those pertaining to treatment, diagnostics, and strain virulence) may become obsolete and require additional updates over time.** Other areas (for instance, fencing suggestions) may be more robust to changing science. To the best of my knowledge, this content is current as of June, 2020.

Thank you for your consideration.

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II. Methods for eradicating *Mycoplasma ovipneumoniae* from domestic sheep or goat operations

Testing efforts in domestic operations

Many state and provincial wildlife jurisdictions are working to build relationships with domestic small ruminant producers, and several partnerships have progressed to the point of testing animals, and considering removal or treatment options. A selection of state-producer partnerships are summarized in Tables 1and 2 below.

Table 1: Collaborative management partnerships. On-going efforts by state wildlife agencies to partner with domestic producers on testing and eventual management of *M. ovipneumoniae* risk from livestock.

Jurisdiction	Operation description	Steps taken	Contact
Nebraska (Pine Ridge)	12 operations	Total of 332 nasal swabs collected to date, testing on-going (x both areas)	Todd Nordeen
Nebraska (Wildcat Hills)	21 operations	Total of 332 nasal swabs collected to date, testing on-going (x both areas)	Todd Nordeen
South Dakota (Badlands)	Goats (86 animals)	Sampled 30 (> 75% of tested animals were PCR-positive for Movi)	Chad Lehman / Austin Wieseler
South Dakota (Badlands)	Goats (20 animals)	Sampled 6 animals (all PCR-negative)	Chad Lehman / Austin Wieseler
South Dakota (Deadwood)	Goats	Tested 7 animals near Deadwood herd; all Movi-negative	Chad Lehman
South Dakota (Rapid City)	Goats/Sheep	Tested 5 goats, 1 sheep near Rapid City; all Movi-negative	Chad Lehman

Follow-up method 1: Test-and-remove

There is increasing evidence from wild sheep that *M. ovipneumoniae* can be locally eradicated, especially from smaller populations, via a protocol of test-and-remove (Garwood et al. 2020; Cassirer et al. in prep, E. Frances Cassirer personal communication). Several attempts have been made to implement a test-and-remove process in domestic sheep and goats with an aim toward *M. ovipneumoniae* eradication, several of which are summarized in Table 2.

Table 2: Test-and-remove projects. Summary information describing a variety of test-and-remove efforts in domestic sheep and goats.

Species (date)	Operation size	Rounds of testing	# Tested/ Positive/ Negative	Age/sex classes included	Who paid	Method for removal	Follow-up testing/ Status

Sheep (~2017)	~50	3 initially,	Unknown	all	IDFG, Rocky Crate	Culled 1 lamb initially, more culls required by subsequent reintroducti on	3 negative whole herd, then owner purchased infected rams to reintroduce
Goats (Jan 2018)	17 (+ 3 kids = 20)	3 (2 for 3 new kids) @ months 1, 2, 3; all qPCR	20/3(+3I)/14 on round 1; on re-test, 20/8/12. All in all, 10 tested positive and were removed; 5 others died	Adults & kids	Alaska Wild Sheep Foundation	Quarantined and evenutally culled	5 remaining goats tested July and were PCR-negati ve. 6 replacemen t animals were also tested, and were all negative
Goats (Jan 2019)	14	3 @ months, 1, 3, 4 (qPCR & cELISA)	14/2/12	Bucks, does, kids	Alaska Wild Sheep Foundation	Both bucks positive and were culled; 3 others died from other causes	9 remaining + 11 kids tested 6 months later (October); all qPCR negative
Sheep (June 2020)	~100	2, but still on-going	Still developing	Ewes, lambs	USFWS/ WSU/ USU	Culled five chronically infected ewes	Planned and in progress

The following points should be considered in the planning stages of any test-and remove effort:

- Who will provide testing supplies and conduct tests, what lab will run the tests, and how will samples be shipped?
- How many animals will be tested, how many times will each be tested, and what are the criteria for determining whether an animal should be removed?
- How will removal occur? Will animals be sold, purchased by a designated buyer for destruction, or destroyed by the producer? If removal is lethal, what methods will be used for carcass disposal?
- Will there be a quarantine period? If so, does the operator have facilities appropriate for quarantine?
- If breeding males are required, how will they be tested? Has the ram provider been contacted, and are they amenable to testing? Will the male(s) be quarantined? How long? If yes, testing can occur well before breeding, but if males are moved from female operator to female operator, testing may need to occur just before the male(s) arrives at the focal operation?
- Will removed animals be replaced, and if so, have available source stocks been identified? What is the testing plan for new arrivals?
- If things don't go as planned, what are the conditions that would cause the project to disband (e.g., more than xx% of sheep pegged for removal, unplanned financial resource gaps, turnover in operator, new disease event in local bighorn herd, etc.)?

- The lambs of the year need special consideration. If there is one or more carrier ewes, it is likely the infection will spread through all lambs, though most will clear by about 1 year of age. Managers and operators shouldn't waste money testing lambs <1 year until efforts are made to remove carrier ewes. Ideally, the operator might be willing to sell all lambs in the fall, then start testing ewes and rams after lambs are sold so as to have three tests completed by lambing.

All of these questions should ideally be addressed ahead of time and written up as a (potentially non-binding) plan, which can be referenced by both the producer and the jurisdiction undertaking the test-remove.

Follow-up method 2: Test-and-treat

Several recent projects have experimented with treating animals infected with *M. ovipneumoniae*. A variety of treatment methods have been investigated, in North America and globally (Table 3). When considering drug treatment regimes, it is important to remember that much of the literature on drug treatments targeting *M. ovipneumoniae* focuses on eliminating clinical signs and lamb weight loss. Complete clearance of *M. ovipneumoniae* is rarely an explicit objective in the agricultural literature, though clearance is the objective in test-and-treat efforts intended to limit risk of *M. ovipneumoniae* spillover.

Four general categories of pharmaceuticals have been considered for treatment of *M. ovipneumoniae*: fluoroquinolones, macrolides, tetracyclines, and lincosamides (Politis et al. 2019). Briefly, the activity of each of these pharmaceutical groups can be summarized as follows (see Politis et al. 2019 for more detail):

- **Fluoroquinolones** (including fluoroquinolones such as enrofloxacin, difloxacin, and marbofloxacin) are broad-spectrum antibiotics, which operate by interfering with bacterial DNA by targeting topoisomerase II (gyrase) and IV. They exhibit good distribution properties in soft tissues, making them amenable for treatment of pneumonia. They are rapidly bacteriocidal, so brief exposures to high drug concentrations may be sufficient to eliminate target organisms.
    - Caveat: fluoroquinolones can incite rapid development of resistance in local bacteria, so they should be used with precaution. Additionally, fluoroquinolones are limited for extra-label drug use in many jurisdictions and banned for use in food-producing animals in several other jurisdictions. They are costly drugs with a relatively high rate of side-effects. In particular they may have effects on connective tissues in growing animals resulting in tendiopathies and arthropathies,precluding their use in lambs destined to become replacement animals (Politis et al.. They often have quite long meat withdrawal times and cannot be used in lactating animals if the milk is for human consumption.
- **Macrolides** (including tilmicosins such as Micotil, tildipirosins such as Zuprevo, tulathromycins such as DRAXXIN, and gamithryomycins such as Zactran) operate by interfering with bacterial protein synthesis, particularly the 50S subunit. They have good distribution in soft tissue. Tilmicosin has been associated with cardiac problems and can be lethal for humans if inadvertently injected via accidental needle stick, but several other macrolides (including tulathromycin) have not. Resistance of *Mycoplasma* spp. to tilmicosin was shown to be high in the mid-2000s (McAuliffe 2005).

Table 3. Summary of drug options for treating *Mycoplasma ovipneumoniae* in domestic sheep.

Drug group	Active ingredient (drug)	Delivery/ schedule	Efficacy in domestic sheep	Licensed for administration in lambs?	Other considerations
fluoroquinolone	Enrofloxacin (Baytril)	Politiset al. 2019 note 3-5 administrations of 5-10 mg/kg body weight (consecutive days)	Note: Goncalves et al. 2010 also suggests enrofloxacin should be effective in goats. Besser et al. (2018) trial showed little effect of injectable Baytril alone.	Yes in some regions. In Canada, label indications for resp. disease in cattle and swine and soft tissue infections in dogs. In Canada, carries a warning against extra-label drug use.	Disallowed in US meat chain. "Adverse effects of fluoroquinolone administration include excretion of yellow faeces and possible damage to connective tissue (e.g., tendinopathies, arthritis lesions), which preclude their use in lambs to be maintained as replacement animals." (Politis et al. 2019).
fluoroquinolone	Difloxacin (Dicural)	Subcutaneous injection 4.0 mg/kg body weight one daily for 3 consecutive days (in lambs in Mavrogianni et al. 2005)	Good -- Mavrogianni et al. 2005. No *M. ovipneumoniae* detected after treatment (at this dosage)		
fluoroquinolone	Marbofloxacin	Subcutaneous injection of 2-3 mg/kg bw (depending on treatment schedule) (Skoufos et al. 2007).	"Two high or three low doses of marbofloxacin, i.e. a total of ≥6.0 mg/kg bodyweight, were needed for effective treatment. Two low doses, i.e. a total of 4.0 mg/kg bodyweight, were not found effective."(Skoufos et al. 2007)		"no local reactions were recorded in any lamb injected with marbofloxacin." (Skoufos et al. 2007)
macrolide	Tilmicosin (Micotil)	Subcutaneous infection with 15 mg/kg bw twice, four days apart (Mavrogianni et al. 2005). Also used in Skoufos et al. 2007, also effective there, also noted emergence of resistance. Politis et al. 2019 note standard dosing recommendation is 2 administrations of 10 mg/dg body weight (consecutive days)	Looked pretty good to me in terms of weight gain, but Mavrogianni 2005 prefers difloxacin. No M. ovi detected after treatment.	Yes in some regions. In Canada, labelled for pneumonic pasteurellosis. Use to clear M. ovi treatment is still considered Extra-label drug use	"It is noteworthy however, that recent papers (Ayling et al., 2000a,b) have reported the finding of tilmicosin-resistant strains of Mycoplasma spp." (from Mavrogianni et al. 2005). Treated sheep must not be slaughtered for use in food for at least 36 days after latest treatment. No use in lactating animals.
macrolide	Tildipirosin (Zuprevo)		Besser et al. (2018) trial showed little effect of injectable Zuprevo alone.		
macrolide	Tulathromycin (DRAXXIN)	"A single dose of tulathromycin (2.5 mg/kg b.w.) was injected subcutaneously" (Naccari et al. 2015)	"In treated animals, the symptomatology decreased rapidly 2 days after treatment and completely after 5-7 days, with remission and normal functioning of respiratory activity." (Naccari et al. 2015). Besser et al. (2018) trial showed some effect on clearing *M. ovipneumoniae* from ewe	Labeled for footrot in sheep in Canada	Naccari et al. 2015. Intl Jo Anim Vet Advances (all I can get is an abstract); Jafari et al. 2016 Iranian Jo Vet Sci and Tech.

			lambs (8 of 18 cleared)		
macrolide	Gamithryomycin (Zactran)		Besser et al. (2018) trial showed little effect of injectable Zactran alone.		
tetracycline	doxycycline				?? I think Mary Wood & Karen Fox have just wrapped up a trial on doxy, but it's not published yet.
tetracycline	Oxytetracycline (Liquamycin)	3-4 administrations of 10 mg/kg body weight (12-hour administration, or 1-2 administrations of long-acting oxytetracycline over 4-5 day intervals at 20 mg/kg body weight) (Politis et al. 2019)		Yes in some regions	"In a recent paper (Ayling et al., 2005), it was described that 60% of >500 *M. haemolytica* or *Mycoplasma* spp. isolates recovered from sheep respiratory disorders around Europe, showed increased resistance to oxytetracycline." Skoufos et al. 2007 discussion. However, Scott 2011 says, "Unlike in cattle, there are few reported oxytetracycline-resistant strains in sheep." Scott later says, "Oxytetracycline (single intramuscular injection of a long-acting preparation at a dose rate of 20 mg/kg BW) should be given to inappetant sick lambs." in his section on treatment of Mycoplasmas.
tetracycline	chlortetracycline				Has been effective against *Mycoplasma hyopneumoniae*
lincosamide	lincomycin	2×5.0 mg/kg body weight (Politis et al. 2019 table 2; cites Skoufos et al. 2006) (or three doses given two days apart for lambs). Intramuscular administration. Politis et al. (2019) note that recommendation is 3 administrations of 5 mg/kg body weight (consecutive day administration)		Yes in some regions.	

- **Tetracyclines** (including doxycycline and oxytetracycline) operate through interference with bacterial protein synthesis, particularly the 30S small ribosomal subunit. These are broad-spectrum antibiotics that exhibit good distribution in soft tissue.
- **Lincosamides** (including lincomycin) should likely be avoided, as they have been associated with mortality when provided in feed to domestic sheep (Bulgin 1988).

Most of these drugs are also effective against *Mannheimia haemolytica* and several other Pasteurellaceae species (*Pasteurella multocida*, *Bibersteinia trehalosi*), with the exception of Lincomycin and Tylosin (Politis et al. 2019).

A set of pilot studies conducted by Dr. Tom Besser examining the capacities of Baytril® (an enrofloxacin), Zuprevo® (a tildipirosin), and Zactran® (a gamithromycin) to clear *M. ovipneumoniae* indicated that injections alone were insufficient to drive clearance (Besser et al. 2018). Draxxin® (a tulathromycin) was effective at reducing *M. ovipneumoniae* load, but effects were inconsistent and many animals remained infected following treatment. An earlier trial with Liquimycin® was also ineffective, though it may have been more successful with adjusted dosing.

Coupling injectable antibiotics with an intranasal antibiotic proved more effective protection, however. In a pilot study, Baytril® injection followed by intranasal Baytril® treatment eliminated carriage from two different domestic ewes. An expanded trial suggested that this effect could be achieved even when the intranasal dosage was low. Baytril® was chosen as an early target drug, since fluoroquinolones exhibit rapid, concentration-dependent bacterial killing properties (it is unclear, by contrast, whether macrolides such as Zuprevo® or Draxxin® are concentration-dependent or time-dependent). Follow-up work on Draxxin® by Drs. Besser and Frances Cassirer indicated that coupling injected Draxxin® with either intranasal Draxxin® or a non-antibiotic intranasal disinfectant (specifically, Povidone or Gentocin) was not as effective at clearance as the Baytril®/Baytril® combination (T. Besser unpublished data).

Extralabel use of fluoroquinolones in food-producing animals was banned in the US by the USDA in 1997, due to concerns that extralabel use of fluoroquinolones in food-producing animals might increase the level of drug resistant zoonotic pathogens and present a risk to public health (https://www.fda.gov/animal-veterinary/antimicrobial-resistance/extralabel-use-and-antimicrobials). In Canada, extra-label use of fluoroquinolones is legal in the context of a valid veterinary-client-patient relationship but is highly discouraged due to the same concerns about the risk of antimicrobial resistance and implications for public health. Health Canada classifies enrofloxacin as a class I antimicrobial, meaning that it is considered of "very high importance" or "critically important" in human medicine, and recommends against extralabel drug use of these products “because it is essential that we protect the efficacy of these drugs by using them prudently and judiciously, as indicated on the approved label” (https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/extra-label-drug-use/questions-answers-health-canada-policy-extra-label-drug-use-eldu-food-producing-animals.html#q16). The Pan-Canadian CVMA guidelines echo these guidelines saying that "class I Antimicrobials by Health Canada should not be used in an extra-label manner in animals destined for the food chain". A veterinarian prescribing Baytril in an extra-label manner takes all responsibility for any adverse events associated with its use. Meat

withdrawal times for enrofloxacin in sheep and goats is unknown and it cannot be used in lactating animals where the milk is destined for human consumption.

Biosecurity surrounding *M. ovipneumoniae* eradication efforts[1]

Biosecurity refers to management measures taken to prevent disease agents from being introduced and spreading to and/or from animal populations or their proximity. Economically speaking, considering biosecurity from the start of an eradication effort is important because it:

- Minimizes risk of new disease,
- Controls and eradicates existing diseases, and
- Increases consumer confidence in the final product.

Biosecurity has three main components: isolation (the confinement of animals away from other animals); traffic control (movement of people, animals, vehicles and equipment); and sanitation/husbandry (cleanliness and care of animals and their environment). Actions and considerations associated with each of these factors are outlined below.

Both test-and-remove and test-and-treat eradication efforts are major undertakings, which should be accompanied by careful consideration of biosecurity to protect the operation against future infection. Operation practices that keep flocks "closed" greatly facilitate biosecurity efforts. In particular, the following should be considered when planning an eradication effort:

- Is fencing adequate to prevent escape by resident animals, or incursion by outsiders?
- Are there other sheep and goats nearby that could escape and come visit resident animals? During the year, do you move your domestic sheep to forage on another farm?
- Is there a testing and quarantine program in place for newly purchased animals?
- If the producer typically brings breeding males in from other sources, is there a plan to test those males, and are back-up males available?
- Is there a plan for follow-up serological testing of new recruits to confirm the effort's success?

Additional considerations are outlined in detail below. These steps, in particular those tied to isolation, are very important, and several eradication efforts have been unsuccessful due to failure to comply with these suggestions.

Isolation: the confinement of animals away from other animals.

The most common way for most new diseases to be introduced into a flock is through new animal additions. Veterinarians typically recommend that new animals and animals returning from exhibitions should be quarantined from resident animals for four weeks to allow for incubation periods of certain diseases. Isolation areas (buildings and pens) should not share the same airspace as resident animals. A distance of 100 feet, if feasible, should separate buildings and pens. The farther away new animals are kept away from resident animals, the better the isolation will be.

[1] This section draws heavily from a guidance document prepared by T. Besser and H. Miyasaki for the American Sheep Industry.

During the isolation period:

- Animals should be observed closely. A veterinarian or health specialist should promptly examine those showing any sign of illness.
- Animals can be tested for specific diseases of concern (*M. ovipneumoniae* and others).
- It is the appropriate time to vaccinate, test, and consider treating for internal and external parasites.
- Other preventative health measures should also be performed during this period.
- All feet should be trimmed, inspected for foot rot and foot bathed in a 10% zinc sulfate solution (Cross & Parker, 1981).
- New purchases should not be allowed to join the resident sheep until they have been tested and proven to be free of drug-resistant (anthelmintic) roundworms (veterinarians can assist with this test, which is also known as the fecal egg count reduction test).

Strict precautions should be taken to avoid spreading potential pathogens:

- Equipment should not be shared between isolated animal areas and resident animal areas.
- People tending these animals should take precautions to avoid spreading disease agents from the isolated animals to other animals and equipment.

Precautions include hand washing, wearing different clothing and footwear, disinfecting feeding and watering equipment and other fomites, and handling animals in quarantine last.

Before adding animals to a flock, remember these principles:

- The health status of the source flock/s should be evaluated. Ask specific questions about the diseases that concern you. Find out specifics about management practices that might affect the flock's health.
- The number of source flocks should be minimized.
- It is best to use a "closed" flock of verifiable good health status as the source for flock additions. A "closed" flock is defined as one where new animals have not been brought in for three or more years. If breeding stock is brought in for genetics or other reasons, isolate and test prior to commingling.

A basic timeline for a test-remove process might proceed as follows:

- Remove chronic carrier ewes, or quarantine them immediately after testing and remove them when their lambs are ~45 days of age.
- Repeat-test all ewes plus a subset (~10 lambs) when the lambs are ~6 months of age in September/October. The lamb tests will provide an indication about whether *M. ovipneumoniae* is still circulating in the flock. The ewe test results can be compared with previous results to identify additional chronic carriers, so this testing may identify additional carriers ewes that need to be culled.
- If the operator does not maintain breeding male, identify the desired source of males, and pre-test a male for use in the breeding season. Depending on the age of the rams, it may be necessary to segregate them, either at the breeder's facilities or locally at the site of the test-remove. The male(s) should be re-tested at the test-remove site just prior to circulating with the ewes.

One plausible timeline for a test-remove effort is shown in Table 4 below.

Table 4. Hypothetical test-remove timeline

Date	Action	Objective	Animals targeted	Samples collected
Winter, 2020	Test ewes	Identify chronic carriers	All adult females	Nasal swabs for PCR
May, 2020	Cull consistently positive ewes	Remove M. ovi reservoir	5 chronically infected ewes	Nasal swabs for PCR
June/July, 2020	Contact breeder re: pre-testing a ram for use in fall	Limit chance of new M. ovi strain introduction during breeding	Ram(s)	Nasal swabs (plus potential segregation of focal ram)
Fall, 2020*	Repeat test most animals	Identify any additional carriers	All ewes plus subset (~10) lambs	Nasal swabs (+blood opportunistically)
Spring, 2021**	Repeat test	Identify additional carriers (especially among new ewes); track antibody declines if no infections are identified	All animals (especially newly recruited females or replacement ewes)	Nasal swabs + blood
June/July, 2021	Ram pre-testing	Limit chance of new *M. ovi* strain introduction during breeding	Ram(s) from operator's preferred source	Nasal swab
Spring, 2022**	Repeat test	Track antibody declines	Subset of ewes, plus all newly recruited lambs	Nasal swabs + blood

*Fall sampling should be scheduled to coincide with either animal handling for lamb sales or some other fall handling activity if possible.

**Spring sampling should be scheduled to coincide with either crutching or shearing, or alternatively when ewes and new lambs are in the jug.

Traffic Control: movement of people, animals, vehicles and equipment

Flock owners and employees should avoid taking biosecurity risks with their own livestock. These include:

a. Exposure of the owner or employees to other flocks or other livestock.

   Be a good neighbor! Don't carry diseases from your place to someone else's place. Avoid unnecessary animal contact when visiting other livestock facilities. Take precautions so you don't carry diseases back to your own place. Change overalls or clothes in between farms. Also either clean and disinfect your boots before entering and when leaving another livestock premises or wear disposable plastic boot covers. Dispose of plastic boots at the farm when your visit is finished.

   Require all visitors to maintain strict sanitation standards. Assess risk factors posed by visitors and take steps to limit their contact with your animals and premises. Do not allow visitors to enter pens or feed alleys, or touch animals unless necessary. Disposable boots or boot washing stations should be available for visitors and required

to be used. Provide visitors with protective coveralls and disposable boots or make thorough boot washing and disinfection required before and after the visit.

b. Poor traffic control (vehicle and personnel) and poor sanitation of vehicles, equipment and clothing may lead to the introduction of disease and is a breach of biosecurity.

   Livestock haulers, feed delivery trucks, dead-stock haulers, etc., should be allowed limited access, and should be held to strict sanitation standards. These standards vary between operations and their physical set-up; however the principles include:

   - Keep visiting vehicles at a distance (and down-wind) from livestock concentration areas.
   - Make separate routes for visiting vehicles versus that farm/ranch's routine livestock and operation traffic, if at all possible and practical.
   - Commercial livestock hauling vehicles should be cleaned and disinfected prior to entering your facility to load your livestock.

Shearing crews should sanitize their equipment between flocks and wear freshly laundered clothing and clean, disinfected footwear. Veterinarians and others who may have close contact with your animals should be very aware of the need for sanitation and take appropriate sanitary measures for their footgear, outerwear and equipment. They should arrive in clean vehicles and wear protective clothing or boots that can be changed or disinfected before leaving.

Sanitation: the practice of maintaining a clean, healthy environment for animals

Keep things clean and picked up. Good sanitation is a necessity in biosecurity.

- Regularly clean and disinfect equipment with appropriate disinfectants;
- Provide proper and timely removal and disposal of manure;
- Provide for the prompt removal and appropriate disposal of dead animals; and
- Control rodents, pests and insects to aid preventing the spread of disease.

Disinfectants are commonly used on vehicles and boots as well as feeding, manure handling and shearing equipment.

- Disinfectants should be used AFTER cleaning the item.
- Disinfectants used should be active in hard water and in the presence of organic material.
- Disinfectants used should be relatively non-toxic and inexpensive, yet effective against a broad spectrum of pathogens.
- The ortho- and chlorophenyl phenols and others meet these criteria, and appear on approved treatment lists. Ensure that any disinfectant chosen is safe for the equipment and environment in which it is used.

Specific practices related to *M. ovipneumoniae*

Isolation

Isolation is the most important aspect of *M. ovipneumoniae* eradication-related biosecurity. The risk of animal-to-animal spread depends on the stage of the infection:

- The time of first infection is the time of highest risk of transmission.
- Most often, this will be recently weaned lambs but potentially could also be adult animals if they had not previously been infected with *M. ovipneumoniae* . Coughing animals are likely more infectious than animals without a cough (Manlove et al. 2017, Besser et al. 2014). Animals likely infected for the first time should be separated at least 15m from other susceptible animals (Felts et al. 2016).
- Segregation must prevent nose-to-nose contacts, but longer separation distances may not be not necessary for these animals.

Traffic control

Traffic control is less important than isolation for controlling *M. ovipneumoniae*. For routine tasks on the farm, handle segregated, *M. ovipneumoniae*-positive (or *M. ovipneumoniae*-unknown status) animal groups only after handling known *M. ovipneumoniae*-free groups on any given day. This is called 'order-of-entry'.

In addition to the general practices described above, shearing crews, veterinarians, brand inspectors, and others who may have close contact with animals should also follow order-of-entry (working *M. ovipneumoniae*-positive/questionable groups only after working *M. ovipneumoniae*-free groups.)

Sanitation

The routine sanitation practices described above are more than adequate to control *M. ovipneumoniae*.

Section II Acknowledgements

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III. History and performance of diagnostic testing for *Mycoplasma ovipneumoniae*

Overview

Diagnostic testing associated with pneumonia in wild sheep has moved through several iterative cycles following shifts in the dominant paradigms surrounding the primary causal agents. Work prior to the 1980s was often built under a paradigm that lungworms were critical causal agents (e.g., Buechner 1960; Forrester 1971); in the 1990s and early 2000s (and

to some extent continuing until today), a suite of members of the Pasteurellaceae family were regarded as critical agents (today, work on Pasteurallaceae often focuses around species that can produce a leukotoxin which can be highly pathogenic in both wild and domestic sheep; e.g., Shanthalingam et al. 2014). *Mycoplasma ovipneumoniae* has been regarded as an important player in this system since the mid-2000s (Besser et al. 2008; Besser et al. 2012; Besser et al. 2013), and is the focus of current diagnostic testing efforts for many jurisdictions. Recent work suggests a potential role for paranasal sinus tumors in propagation or carriage of *M. ovipneumoniae* (Fox et al. 2011; Fox et al. 2015), and on-going field efforts in several states aim to document attributes of these tumors and link them to demographic responses in particular bighorn herds. Here, we briefly review the history of diagnostic testing for *M. ovipneumoniae*, and end with some suggested guidelines to demonstrate freedom from disease in a flock or herd, as well as freedom of disease in an individual wild or domestic sheep or goat.

Diagnostic testing protocols for *M. ovipneumoniae*

Diagnostic tests for most pathogens take on two basic forms. A test can either aim to detect direct evidence of the pathogen itself (DNA-, antigen- or growth-based tests), or it can aim to detect antibodies that the host produces in response to a particular pathogen (these are serological tests).

Serological testing for evidence of past exposure to *M. ovipneumoniae*

Serological tests assess evidence of past exposure to a pathogen through antibodies. Two different serological tests have been used for *M. ovipneumoniae* in caprine hosts. The first is an indirect hemagglutination assay (IHA) test, which was commonly used for *M. ovipneumoniae* serology through 2008 (Black et al. 1988; Cho et al. 1976). The IHA test proved difficult to control due to variability between antigen preparations, and was discontinued by the Washington Animal Disease Diagnostic Lab (WADDL) when the competitive enzyme-linked immunosorbent assay (cELISA) test was developed.

The second serological test, which is the one currently suggested by WADDL, is a competitive ELISA test. The test is based on the ability of serum antibodies to compete (displace) binding of a monoclonal anti-*M. ovipneumoniae* antibody. The monoclonal antibody binds to an immunodominant (in bighorn sheep) surface-exposed antigen. It is currently not known whether the antibody detected by this test is capable of neutralizing *M. ovipneumoniae.* However, domestic sheep immunized with killed *M. ovipneumoniae* whole cells became positive on the cELISA test and also developed neutralizing antibodies against *M. ovipneumoniae* (Ziegler et al. 2014) cELISA test results are reported as both categorical and quantitative measurements. The quantitative measurement is reported in terms of percent inhibition (%I). Higher %I values correspond directly to better ability of the serum to block binding of the monoclonal antibody used in the test, and is presumed to indicate a higher degree or duration of immune system stimulation. WADDL currently classifies %I values above 50 as seropositive, %I between 40% and 50% as indeterminate, and %I values below 40% as seronegative.

Direct testing to detect active *M. ovipneumoniae* infections

Early direct diagnoses of *M. ovipneumoniae* were typically based on growth of the microorganism, and then identification of species based on colony morphology (e.g., Black et al. 1988). PCR-based diagnostic methods detect presence of an organism's genetic material directly (thus an individual who had an infection, but has cleared it, should not test positive by PCR-based methods). PCR-based methods for detecting *M. ovipneumoniae* have been refined at several points over the last twenty years. We briefly review those refinements, and attempt to clarify the utility of several approaches in current use.

Original PCR-based diagnostic testing for *M. ovipneumoniae* relied on amplification of a segment of the 16S small ribosomal subunit. This region of the bacterial genome is widely used for identifying bacteria to the species level (for instance, it is regularly used as a target region for bacterial microbiome research). The 16S region is particularly useful because it contains DNA sequences ranging from those broadly conserved across all bacteria to those highly specific to single bacterial taxa. A 16S-based PCR assay for detection of *M. ovipneumoniae* in domestic sheep was first put forward by McAuliffe et al. in 2003; to increase test specificity, McAuliffe's test also required detection of a specific restriction pattern of the PCR product. Subsequently, Besser et al., 2008 applied the McAuliffe test to detect infected bighorn sheep. These PCR tests were 'conventional' PCR, in which all amplification cycles were run prior to any measurement of PCR products (typically done by separation on agarose gels stained by ethidium bromide). As a consequence, these methods did not provide information on the starting number of gene copies (the pathogen "load") in a particular sample; they could simply detect whether the organism was present.

Real-time PCR methods for detection of *M. ovipneumoniae* were developed by WADDL, and first reported in Lawrence et al. (2010). The real-time PCR originally targeted a subset of the same 16S region of the bacterial genome, and included one of the McAuliffe primers, a new second primer that reduced the amplicon size, and a TaqMan probe, enabling recording of amplicon generation in "real time" so that the particular amplification cycle in which fluorescence passed a specified threshold was recorded. Real-time methods provide more specificity (since both primers and the probe must be homologous to the target DNA sequences) and may be used to develop quantitative information (gene copy number of the organism in the original sample) if suitable controls are included.

The real-time methods were refined by Besser et al. 2012, who added an additional locus (capturing DNA from a portion of the 16S-23S intergenic spacer region) into the real-time protocol.

In 2016, Walsh et al. conducted a ring test, where replicate aliquots of a set of samples were sent to multiple labs, to determine lab-to-lab consistency in *M. ovipneumoniae* PCR detection protocols. Participating labs included WADDL, Wyoming Game and Fish, Dr. Tom Besser's laboratory, Dr. Glen Weiser's laboratory (which employed a version of the McAuliffe protocol that differed slightly in amplification conditions from those used by Dr. Besser and WADDL), Dr. Srikumaran Subramaniam's lab, and the Colorado Parks and Wildlife laboratory. All six labs generally agreed in their ability to detect *M. ovipneumoniae* DNA, though there was some disagreement between samples with very low loads about whether samples were classified as indeterminate or positive (Walsh et al. 2016).

Table 5. PCR target loci for Multilocus Strain Typing.

Locus	Coding/ non-coding	Reference for 1st inclusion	Reason for inclusion	M. ovi detection methods that include this locus	Multiple primer options?	Notes
16S	Non-coding	McAuliffe et al. 2003	Standard locus for bacterial species identification	Universal Method, Laura McAuliffe-based methods, current WADDL methods, most other labs	Yes. Universal method relies on one set of primers, most other diagnostic labs rely on the Laura McAuliffe ("LM") primer set (McAuliffe et al. 2003).	There are at least two different sets of primers that get used to isolate the 16S region: the "universal" primer set, and LM (Laura McAuliffe) set. The "universal primers" are thought to have low sensitivity and specificity for *M. ovipneumoniae* (though they do perform well for other Mycoplasmas), so WADDL advises against their use.
16S-23S intergenic spacer (IGS)	Non-coding	Besser et al. 2012b	This region is variable among *M. ovipneumoniae* isolates, and allowed for initial efforts at strain identification	All (current and historic) strain-typing efforts, current WADDL methods	Not currently.	Early on, strains were referenced by the length of their IGS sequences, since strains typically varied by indels in IGS (e.g., "Strain 404" in Cassirer et al. 2017 had an IGS region of length 404 bp). However, this practice has largely been replaced with the advent of Multi-locus strain typing (MLST) methods that use the 16S, *rpoB*, and *gyrB* regions in addition to the IGS region.
rpoB	Coding	Cassirer et al., 2017	Housekeeping gene; codes RNA polymerase B	Current WADDL strain-typing methods	Not currently.	"Housekeeping genes" are expressed at relatively constant rates under a wide range of physiological conditions.
gyrB	Coding	Cassirer et al. 2017	Housekeeping gene; codes gyrase B	Current WADDL strain-typing methods	Not currently.	

Following the ring test, methods were modified again by Cassirer et al. 2017 to include two additional house-keeping genes (*gyrB* which codes for a gyrase, and *rpoB*, which codes for a reverse transcriptase). This expansion was done in part to facilitate identification of specific strains. The methods presented in Cassirer et al. 2017 were used by Kamath et al. 2019 without revision, and are the current protocol used by WADDL for strain typing. A summary of loci used in the MLST methods is included in Table 5.

In 2019, Dr. Maggie Highland's USDA-ARS lab used an alternative approach in an effort to identify additional amplification sites to further refine the amplification protocols so as to limit the number of false negatives (and false positives) detected under the currently WADDL protocol (Highland et al. 2018).

Diagnostic test performance

PCR test

Both the antibody (cELISA) and direct (PCR) test for *M. ovipneumoniae* are quantitative (or semi-quantitative in the case of the PCR) tests, which is to say, the test produces a continuous metric that is then discretized into categories of positive/indeterminate/negative. The quantitative values can provide some additional insight into the strength of a particular animal's antibody response (for the cELISA), or the extent of its infection (for the real-time PCR). The cut-off values associated with the different categorical outcomes are determined by WADDL and other diagnostic labs, and reflect their best views on pathogen dynamics.

Table 6. Diagnostic testing methods for detecting *M. ovipneumoniae*.

Test	Target	Quantitative measurement	Range of possible values	Cut-off value for indeterminate / positive	Basis for cut-off
Real-time PCR	Pathogen DNA in the host	Cycle threshold (Ct): number of amplification cycles required for fluorescence to cross the set threshold value. Lower values equate to a higher *M. ovipneumoniae* load in the sampled animal.. 40 = 0 M. ovi gene copies in the starting sample, 39 = ~1 gene copy, 38 = ~2 copies, 37 = ~4, 36 = ~8, 35 = ~16, etc.	0-40 (40 = no *M. ovipneuoniae* detected)	40 = negative; 37-39 = indeterminate; <36 = positive	Some false amplification can occur if primers bind to related, non-target sequences. This is rare, and inconsistent, thus the low cut-off values.
cELISA	Antibodies to the pathogen in the host	% inhibition.	0-100	0-39.9 = negative; 40-49.9 = indeterminate; 50-100 = positive	Cut-offs were determined using antibodies from animals who had survived a selection of the very severe pneumonia events in the winter of 2009.

There is good evidence that the false-positive rate of the current WADDL PCR methods for *M. ovipneumoniae* detection are quite low now, and have been low for the last decade. Manlove et al. (2019) analysed a set of 242 *M. ovipneumoniae* samples from domestic sheep in the U.S., ran all samples through the WADDL PCR process, and also sequenced all

PCR products. In that effort, all samples produced genetic sequences consistent with *M. ovipneumoniae* sequences stored on GenBank, suggesting that the WADDL PCR produced 0 false positives out of 242 trials. Furthermore, the WADDL PCR results run in 2018 matched WADDL PCR results from 2011 in 369 of 370 cases, suggesting that the false-positive rate of the WADDL methods has been quite low for the last decade. AHC runs PCR testing for *M. ovipneumoniae* using a protocol very similar to that of WADDL. The AHC protocol consists of sequencing the real time PCR product (~150 bp) from a smaller subset of samples for confirmation (unless the submitter requires sequence confirmation for all positives). The diagnostic specificity for domestic sheep and goat is approximately 96% based on results from 95 domestic sheep and goat samples tested between Aug 2019 and July 2020. False positives typically arose due to cross-amplification of *Mycoplasma bovoculi* or *Mycoplasma conjunctiva* (Tomy Joseph personal communication; Table 7).

Table 7. *M. ovipneumoniae* PCR test performance at AHC (data provided courtesy of Tomy Joseph).

Host species	Total sequenced	# True positives	# False positives	# suspect samples (Ct > 36)
Sheep	75	73	2	11
Goats	20	18	2	3

False-negative rates on the *M. ovipneumoniae* PCR could arise from several sources, and the likelihood of false-negative results remains somewhat unclear. False-negatives could arise via (at least) the following four routes:

1) Swabbing efforts in infected animals that fail to pick up the organism (i.e., "miss" capturing *M. ovipneumoniae* from the animal's nostrils). Polyurethane culture swabs (BD CultureSwabTMEZ System or similar) are strongly preferred. WADDL advises practitioners as follows: "Use swabs made of synthetic materials (tip and shaft)." Some detection issues can be mitigated through bilateral sampling (Felts 2020 Chapter 3), since some animals do appear to have entirely unilateral infections (Felts 2020 Chapter 3). Handlers should be aware that detection probabilities may decline through serial swabbing (e.g., if multiple rounds of swabbing are conducted in a single handling event, the probability of detection will likely be highest on the first swab, and this is particularly true for unilateral sampling, Felts 2020 Chapter 3).
2) Transport protocols that allow for inadvertent degradation of *M. ovipneumoniae* within the sample, so that while the sample originally contained *M. ovipneumoniae*, the organism was no longer detectable once the sample arrived at the lab. WADDL advises: "...Avoid swabs with agar-based transport media, as these are inhibitory to PCR. Swabs may be submitted dry in their sheath or other sterile containers, or may be placed in glycerol-containing media; swabs should be held frozen if delays are expected prior to testing."
3) Diagnostic testing (i.e., amplification) protocols (including primers, cycling conditions, and plating conditions) that fail to give rise to a "detectable" level of *M. ovipneumoniae*, even when *M. ovipneumoniae* is present in the sample.
4) Polymerase inhibitors from the nasal swab sample carried over to the PCR tube.

There is reason to believe that false negatives do sometimes arise. For example, Butler et al. (2017) used a Beta distribution to describe detection probabilities (at the population level) for *M. ovipneumoniae* using two different transport protocols (dry swab and a swab stored in TSB), and found mean detection probabilities (again, at the population level) of 63.0% for the dry swab, and 72.4% for the TSB transport. However, both methods were rarely applied to the same populations or individuals, confounding direct methodological comparisons, and furthermore, the best practice for detection of *M. ovipneumoniae* circulation at the population level is thought to be derived from an antibody (i.e., cELISA-based) test (Cassirer et al. 2018).

Table 8. Detection of *M. ovipneumoniae* in wild sheep populations (values estimated by Butler et al., 2017)

Transport / amplification method	Butler posterior estimates	Mean detection probability (alpha / (alpha + beta)	Variance
qPCR	Beta(6.28, 3.69)	63.0%	0.0213
TSB	Beta(60.18, 22.91)	72.4%	0.0024

One important consideration when assessing PCR-based tests is the possibility of amplicon contamination in the testing lab. Accredited labs are assessed regularly in terms of their ability to limit contamination, thus it is often advisable to use an accredited lab for PCR-based diagnostics (D. Walsh, personal communication). Currently, WADDL is the only accredited diagnostic laboratory for testing for *M. ovipneumoniae* in the U.S. The AHC lab is accredited and conducts *M. ovipneumoniae* PCR testing in Canada, though no Canadian labs currently conduct serological testing. Nonetheless, labs do typically agree strongly in their PCR-based *M. ovipneumoniae* diagnostics. Performance in the Walsh study did not appear to vary between direct extraction and enrichment protocols. None of the labs detected *M. ovipneumoniae* in any of the negative control specimens, suggesting the false positive rate may be relatively low.

Serological test

The cELISA test is intended for interpretation at the population level. The sensitivity of the cELISA test offered by WADDL is proprietary and not readily available, however, the test has been so widely used in the bighorn sheep community that some basic lines of evidence have emerged.

It appears that the cELISA test very rarely (if ever) produces herd-level results indicating infection when no history of disease has been documented (there is little-to-no evidence of false-positives on the serological test, though epidemiological history for many animals is unclear and the possibility of false positives cannot be entirely ruled out). There is some indication that false negatives may arise in certain contexts (e.g., if the animal has only recently been exposed and has not yet mounted a detectable antibody response, but also in cases where the infecting strain or infected operation does not incite or mount a detectable response; Johnson et al. 2020). Additionally, the cELISA test does not perform well in domestic goats, and is not recommended for use in that species. A study of *M. ovipneumoniae* prevalence and seroprevalence in U.S. domestic sheep operations found some discrepancies between PCR-based and ELISA-based results at the operation level (Manlove et al. 2019). A substantial

proportion of operations were either sero-positive or PCR-positive, but not both. Moreover, recent assessment of serially sampled cELISA results from several relatively low-severity *M. ovipneumoniae* establishment events in bighorn sheep have shown whole populations in which antibody expansion is very low and tests continue failing to meet the benchmark for being classified as "seropositive", even when active infection has been present for several years.

Taken together, these two observations suggest a need for longitudinal studies of the antibody dynamics of *M. ovipneumoniae* within particular host animals to add additional clarity for interpretation of the serological test results.

Interpretation of the cELISA results at the individual level is somewhat more equivocal (and explicitly not recommended by WADDL).

Diagnostic testing guidelines

The WAFWA Wildlife Health Committee (2015) provided the following recommendations for determining *Mycoplasma ovipneumoniae* status of bighorn herds:

- Minimum sample sizes for surveillance should be 10% of herd or sub-herd
- Serum banking is recommended for all herds, regardless of their apparent health status.
- Collect serum for *M. ovipneumoniae* serology, and nasal swabs, and tissue samples (post-mortem) for *M. ovipneumoniae* PCR. In particular, *M. ovipneumoniae* may be detectable in samples of consolidated lung tissues and along the leading edge of the leading edge of necrotic tissue, and also on bronchial junction swabs.
- *M. ovipneumoniae* strain typing is recommended, but not assertively advocated
- Nasal swabs should be collected using sterile Dacron swabs with plastic stems. Collect the samples by gently inserting the swab deep into each nostril while rotating swab. Do not touch the outside of the nares during sampling
- Swabs should be placed in a dry cryovial, Mycoplasma enrichment media, or TSB/glycerol. Insert the tip of the swab into the vial, then break off the stem and replace the cap on the tube. Transport media decisions will depend to some extent on the goal of the sampling. For whole genome sequencing, for example, TSB/glycerol or mycoplasma broth seem helpful. For standard PCR diagnostics, dry swabs are sufficient, at least for WADDL.
- Keep samples cool during collection and shipment. Samples collected in TSB/glycerol should be frozen, and can then be held indefinitely. However, they must remain frozen during shipping, which may require use of dry ice.

We suggest the following refinements to these recommendations

- Strain type at least one sample per novel disease event. If a herd is highly compartmentalized and experiencing asynchronous disease dynamics, consider strain typing a sample from each subunit.
- WADDL will now accept dry nasal swabs, however, TSB/glycerol may still be useful if whole genome sequencing of the *Mycoplasma* strain is desired.
- For post-mortem detection of *M. ovipneumoniae* in the lower respiratory tract, deep swabs of the three major bronchi seem to be more sensitive than lung tissue samples.

Section III Acknowledgements

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IV. Host range and potential role of non-caprinae species in epidemiology of *M. ovipneumoniae*

Fewer than 10 tests of camelids have been conducted by WADDL as of a query on March 15th. The AHC has tested 14 llamas as of March 2020, and all were negative for *M. ovipneumoniae*.

Table 9: Number of animals tested for M. ovipneumonia from British Columbia by the Animal Health Centre as of March 4, 2020.

Species	Number of animals from BC tested
Bighorn sheep	265
Caribou	180
Thinhorn	105
Elk	0
Llama	14
Moose	140
Mountain goat	134
Mule deer	0
Muskoxen	0
Domestic Goats	370
Domestic sheep	1204

Testing results from within British Columbia for a range of potential host species are included in Table 9. These data are imperfect as a reflection of prevalence in domestic animals as coughing is often the impetus to test for *M. ovipneumoniae*, which would bias the sample towards being positive. Some of these samples were obtained through trials testing Baytril or other treatments, or test and cull programs as ways of eliminating *M. ovipneumoniae*, which could again bias estimated prevalences. prevalence.

V. Relative risk and clinical significance of *M. ovipneumoniae* transmission from domestic goats as compared to domestic sheep.

Prevalence of *M. ovipneumoniae* in domestic goats

M. ovipneumoniae can infect, and is sometimes associated with severe pathology in, domestic goats (e.g., Goncalves et al. 2010, Rifatbegović et al. 2011, Akwuobu et al. 2016). Currently,

information on infection prevalence and consequences in domestic goats is relatively sparse, but more information may be available on prevalence of *M. ovipneumonaie* in domestic goats following completion of the USDA-APHIS-CEAH-NAHMS Goat 2019 study (USDA-APHIS-NAHMS 2019a, b). A public outreach and education study in three focal areas of Washington State found a mean overall *M. ovipneumoniae* prevalence of 28%, and a mean prevalence of 58% among infected operations in a sample of 84 goats from 16 private-land flocks (Heinse et al. 2016). However, the uncertainty associated with those estimates is quite high, and the results may be specific to the areas where the study took place. It is thought that most goats testing PCR-positive on nasal swabs appear healthy, though more formal data on this front would be helpful.

Somewhat more generalizable information is available specifically for pack goats. Dr. Margaret Highland (Kansas State University) conducted a study of pack goats, and goats housed with pack goats, covering 576 animals from 83 premises across 13 states. *M. ovipneumoniae* was detected by Dr. Highland's lab from 30 animals, localized to 5 of the 83 sampled premises (with herd sizes of 7 to ≥15 goats per premise), indicating much lower infection rates at both the operation- and the individual-level than has been reported in domestic sheep (e.g., Manlove et al. 2019). Furthermore, the preponderance of the goats that tested positive for *M. ovipneumoniae* were under 1 year of age (with 23 under 5 months of age).

Table 10. *M. ovipneumoniae* prevalence in pack goats (data provided courtesy of Dr. Margaret Highland).

	% positive	**# positive**	**# sampled**
Overall	8.1	46	571
Under 4 months age	23.8	25	105
Over 4 months age	4.5	21	466
Packers over 4 months of age	5.1	19	371
All over 1 year of age	4.4	19	428
Packers over 1 year of age	4.9	17	346

Virulence of goat-clade *M. ovipneumoniae* strains

Kamath et al. (2019) include data on 34 strains of *M. ovipneumoniae* collected from domestic goats. All but one of those strains were in the "goat clade" of the *M. ovipneumoniae* phylogeny. This genetic separation among domestic sheep and domestic goat strains is consistent with results from Europe, which also found complete separation of *M. ovipneumoniae* strains into host-specific clades (Maksimovic et al. 2016).

There is limited comparative work on the virulence of goat- vs. sheep-clade *M. ovipnuemoniae* strains in bighorns, but several well-documented case studies suggest that goat-clade strains may be less virulence. Cassirer et al. (2017) documented invasion of a novel goat-clade *M. ovipneumoniae* into a previously infected bighorn sheep herd. That herd exhibited a 38% mortality rate (5 of 13 animals) among adults, which aligns well with mortality rates observed by Foreyt and coauthors (2009) in a goat - bighorn commingling study intended to document transmission of lungworms. A captive study indicated that direct contact with

domestic goats harboring goat-clade *M. ovipneumoniae* strains caused sublethal pneumonia in bighorn sheep (Besser et al. 2017). Recently, a goat-clade strain invaded the Rio Grande Gorge Rocky Mountain bighorn sheep herd, and is currently being investigated by the New Mexico Department of Game and Fish, and Taos Pueblo Tribe, and Utah Division of Wildlife Resources. To date, that strain has produced no mortality among approximately 20 free-ranging, infected, instrumented bighorn sheep.

All of those findings focus on disease-induced mortality among adults, and less is known about the burden that goat-clade strains place on bighorn lambs. This is a critical knowledge gap, since long-term lamb mortality burden is the major impediment to bighorn population recovery following *M. ovipneumoniae* invasion (Cassirer & Sinclair, 2007; Manlove et al. 2016). New Mexico Game and Fish and the Taos Pueblo Tribe will examine lamb survival in the presence of a goat-clade strain as part of their new study and this project may begin to fill that gap.

Risk of encounter between domestic goats and bighorn sheep

Goats are numerically less common, and typically managed in smaller operations, than domestic sheep (in the US in 2017, 5.20 million sheep were distributed across 88,338 operations, whereas only 2.64 million goats were spread across 128,458 operations, USDA-APHIS-CEAH-NAHMS 2016). In the US, goats are highly regionally concentrated, with the preponderance of goat production in Texas and the eastern United States (https://www.aphis.usda.gov/animal_health/nahms/smallscale/downloads/Small-scale_goat.pdf pg 4). There is an important cluster of angora goat production in and around the Navajo Nation in the Four Corners area, and dairy goat production is proportionally somewhat higher in regions of the intermountain west (https://www.aphis.usda.gov/animal_health/nahms/smallscale/downloads/Small-scale_goat.pdf). There are clusters of dairy goat production near Reno, NV; Spokane, WA; and Salt Lake City, UT, which may be relevant concerns for bighorn management in those areas. According to a search of the Bureau of Land Management's Rangeland Administration System system (accessed by K. Manlove on 2020-06-08), there are currently 30 grazing allotments dedicated to goats in the US (2 each in Arizona and Colorado, 1 in Montana, 17 in New Mexico, 3 in Nevada, and 5 in Wyoming), totalling less than 3,500 animal unit months per year (Table 11).

Goats are conventionally divided into at least three management groups -- meat, dairy, and fiber goats -- and it may be useful to regard risk arising from each of these groups separately. A study by Drew & Weiser (2017) found that pack goats were more frequently vaccinated, and bore lower pathogen burdens, than meat goats among a set of goat operations from Idaho. Pack goats are often intensively managed, and are regarded as pets by their owners. These observations, coupled with apparently limited infection among pack goats (Table 10) suggests they may pose a more limited risk to bighorn sheep.

Aggregate risk of pack goats and meat goats to bighorn sheep

Taken together, these data provide some suggestion that domestic goats likely pose a substantially lower risk of *M. ovipneumoniae* transmission and associated disease burden on bighorn sheep than do domestic sheep. Goats -- and particularly pack goats -- tend to exhibit a

Table 10. Domestic goat grazing allotments. US BLM goat grazing allotments (from BLM-RAS accessed 2020-06-08).

Field Office	Auth No	Permit Status	Allotment Name	Livestock Number	Allotment Num	Period Begin Date	Period End Date	Type Use	Public Lnd Pct	AUMs
KINGMAN FO	202272	FLPMA 402(C)(2)/APPROP ACT	MT. TIPTON	35	AZ00058	2013-03-01	2014-02-28	ACTIVE	96	81
HASSAYAMPA FO	201215	FLPMA 402(C)(2)/APPROP ACT	DEWEY	75	AZ06094	2000-03-01	2001-02-28	ACTIVE	100	180
LITTLE SNAKE FO	501212	FULLY PROCESSED	UPPER TROUT CREEK	46	CO04169	2019-05-15	2019-11-30	ACTIVE	100	60
LITTLE SNAKE FO	501215	FLPMA 402(C)(2)/APPROP ACT	TROUT CREEK	194	CO04170	2020-05-15	2020-11-30	ACTIVE	40	102
BUTTE FO	2507709	FULLY PROCESSED	SIEBEN	33	MT07709	2009-05-15	2009-12-31	ACTIVE	100	50
FARMINGTON FO	3000151	FLPMA 402(C)(2)/APPROP ACT	WEST HEAD CANYON	4	NM05147	2017-09-12	2018-02-28	CUSTODIAL	100	4
FARMINGTON FO	3025839	FLPMA 402(C)(2)/APPROP ACT	EAST HEAD CANYON	15	NM05146	1990-03-01	1991-02-28	ACTIVE	100	36
ROSWELL FO	3000771	FULLY PROCESSED	ARROYO SECO NORTH	5	NM63047	2016-03-01	2017-02-28	ACTIVE	61	7
ROSWELL FO	3001458	FULLY PROCESSED	COWBOY MILL RANCH	5	NM63034	2015-03-01	2016-02-28	ACTIVE	63	8
ROSWELL FO	3006027	FULLY PROCESSED	SALT CREEK	350	NM64021	2014-03-01	2015-02-28	ACTIVE	53	445
ROSWELL FO	3006029	FULLY PROCESSED	JONES-EAST CEDAR HILL	150	NM64028	2012-03-01	2013-02-28	ACTIVE	45	162
ROSWELL FO	3006060	FLPMA 402(C)(2)/APPROP ACT	CHIMNEY CANYON	100	NM64030	2010-03-01	2011-02-28	ACTIVE	54	130
ROSWELL FO	3006089	FULLY PROCESSED	LUCKY LAKE	6	NM64065	1989-03-01	1990-02-28	ACTIVE	19	3
ROSWELL FO	3006100	FULLY PROCESSED	CEDAR HILL	100	NM63048	2013-03-01	2014-02-28	ACTIVE	70	168
ROSWELL FO	3006101	FULLY PROCESSED	CHINA DRAW	100	NM64070	2010-03-01	2011-02-28	ACTIVE	55	132
ROSWELL FO	3006175	FULLY PROCESSED	MACHO	411	NM64008	2013-03-01	2014-02-28	ACTIVE	65	641
ROSWELL FO	3024544	FULLY PROCESSED	THE BANK RANCH	10	NM62077	2012-03-01	2013-02-28	ACTIVE	100	24
ROSWELL FO	3026917	FULLY PROCESSED	TRI COUNTY RANCH	89	NM62049	2014-03-01	2015-02-28	ACTIVE	35	75
CARLSBAD FIELD OFFICE	3001121	FULLY PROCESSED	LAST CHANCE CANYON	10	NM78116	2014-03-01	2015-02-28	ACTIVE	90	22

CARLSBAD FIELD OFFICE	3001220	FULLY PROCESSED	DEER CANYON	150	NM78048	2003-03-01	2004-02-28	ACTIVE	87	313
CARLSBAD FIELD OFFICE	3020172	FLPMA 402(C)(2)/APPROP ACT	SOUTHEAST JAL	5	NM76054	2007-03-01	2008-02-28	ACTIVE	100	12
CARLSBAD FIELD OFFICE	3027083	FLPMA 402(C)(2)/APPROP ACT	LITTLE BOX CANYON	10	NM78062	1989-03-01	1990-02-28	ACTIVE	86	21
BRISTLECONE FO	2703636	FLPMA 402(C)(2)/APPROP ACT	BECKY CREEK	377	NV00404	2017-11-01	2018-03-15	ACTIVE	100	335
BRISTLECONE FO	2703636	FLPMA 402(C)(2)/APPROP ACT	LOVELL PEAK	86	NV00406	2017-07-01	2017-09-30	ACTIVE	100	52
BRISTLECONE FO	2703636	FLPMA 402(C)(2)/APPROP ACT	WHITEMAN CREEK	96	NV00408	2017-05-01	2018-02-28	ACTIVE	100	192
RAWLINS FO	4903412	FLPMA 402(C)(2)/APPROP ACT	THE BUTTES	1	WY09079	2001-05-01	2001-11-01	ACTIVE	32	1
RAWLINS FO	4903452	FLPMA 402(C)(2)/APPROP ACT	BUFORD	39	WY09130	2006-03-01	2007-02-28	ACTIVE	41	38
CASPER FO	4906002	FLPMA 402(C)(2)/APPROP ACT	ALLEMAND	308	WY10071	1999-03-01	2000-02-28	ACTIVE	16	118
CASPER FO	4906666	FLPMA 402(C)(2)/APPROP ACT	TTT-SCOTTS PLACE	12	WY10139	2000-07-01	2001-02-28	ACTIVE	57	11
LANDER FO	4903839	FLPMA 402(C)(2)/APPROP ACT	ATLANTIC CITY COMMON	12	WY01901	1994-05-20	1994-09-30	CUSTODIAL	83	9

lower prevalence of *M. ovipneumoniae* (see Table 10), and when they are infected, they often carry strains of apparently lower virulence. Pack goats are intensively managed when transiting bighorn sheep ranges, and their management likely further reduces the risk they pose to bighorn. Backyard and meat goat herds may pose higher risks, and operators should take precautions to avoid contact between their goats and bighorn sheep. Many state agricultural extension offices now emphasize the need to actively manage grazing and pastured animals (e.g., Chapman, K. 2018), and concerns about disease risk for goats are articulated at several points in the USDA-APHIS literature.

Section V Acknowledgements

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VI. Fencing to limit risk of *M. ovipneumoniae* transmission

Fencing to limit host movements

Fencing, especially along highway corridors, can constrain bighorn movement across the landscape (e.g., Epps et al. 2005, Epps et al. 2007), but fencing that completely eliminates movement of keen jumpers like bighorn sheep is relatively rare, especially over decadal timescales (Epps et al. 2018). Even after a preliminary study showed complete removal of movement by radiocollared animals over 3 years, gene flow and apparently also disease transmission occurred a decadal timescale in one desert bighorn system (Epps et al. 2018). Mooring et al. (2004) claimed that a 2.6m woven game fence precluded bighorn movements in and out of the Red Rocks Wildlife Area in New Mexico. Epps et al. (2005) showed that fencing (coupled with busy highways) had apparently been sufficient to reduce gene flow among bighorn sheep herds in the western Mojave. A project in Washington state produced somewhat more equivocal results. There, a highway fence was built to keep cervids and bighorn sheep off a US highway in 2013. In that project, an 8-foot steel fence was constructed to limit animal visits to the road. While the project did reduce bighorn sheep vehicle collisions on US Hwy 97 Alternate Route from over 5 animals per year prior to fence construction, an average of 1.8 occurrences continued to occur each year in the ten years following fence construction (Kelly McAllister, personal communication).

Fencing can also constrain movements of domestic sheep, and improving fencing infrastructure is a relatively popular option for producers. In an interview-based study of 40 small-scale producers around Washington State, Heinse et al. (2016) found that 94% of producers "kept animals contained within fences". 25 of 34 responding producers in that study reported that some animals had escaped their fences. In 12 cases, those animals left the premises entirely, and in four instances, the animals -- all goats -- never returned. Heinse et al.

additionally found that installation of fence, construction of internal isolation pens, and double-fencing were all popular management actions (83-100% of respondents said they would undertake these actions) among the small-scale producers they studied, suggesting producers may generally have a positive attitude toward fencing as an intervention for transmission.

A summary of efforts to build bighorn sheep-proof fence and their effectiveness is included in Table 12.

Separation zones and transmission distances

Single perimeter fences can still allow for fenceline contact, which may be sufficient for transmission in some cases. A number of fenceline contact experiments between wild and domestic sheep have been published (Foreyt & Jessup 1982, Foreyt 1989, 1990, 1994, Onderka & Wishart 1988) and extensively reviewed (Wehausen et al. 2011) in the existing literature. Though many of these studies preceded recognition of *M. ovipneumoniae* as a critical player in the system, they still provide clear evidence that fenceline contact can allow for transmission of pneumonia-causing agents between domestic and bighorn sheep, regardless of the particular agents at play. Whether fencing alone can preclude transmission for animals along a fenceline likely depends on 1) infection prevalence of animals at the fence; 2) symptom production of animals at the fence; 3) duration of exposure (i.e., how long animals aggregated along the fenceline).

Like most mycoplasmas, *M. ovipneumoniae*, generally has limited environmental persistence due to its lack of a cell wall. Transmission is thought to occur primarily through respiratory droplets, with force varying among infected animals (see for example Manlove et al. 2017). There is some evidence that force of infection arising from animals that are not showing signs of shedding (nasal discharge) or respiratory disease (coughing) may be relatively low, and that fairly intensive contact is required to spark transmission (Besser et al. 2014). Dosing from asymptomatic domestic sheep (or bighorn sheep) may be relatively low, and data from bighorn sheep indicate that free-ranging infected animals do not always transmit the pathogen on to lambs when contact rates are low (Cassirer et al. 2013; Manlove et al. 2017). If rapid testing is available, it may be desirable to hold captured foraying bighorns overnight for testing.

Intensive contact is not always required for transmission, however. *M. ovipneumoniae* is highly infectious when clinical signs are present and has been observed to transmit rapidly even in the absence of direct contact (Besser et al. 2014; Felts 2020 Ph. D. thesis Epidemiological investigations of bighorn sheep respiratory disease and implications for management). For instance, in a captive study at South Dakota State University, transmission occurred between acutely infected and coughing bighorns in pens separated by approximately 50m (Brandi Felts et al. in prep).

Single perimeter fences

Single-perimeter fences provide a barrier, albeit a penetrable one, to bighorn movement. Recent wildlife fencing projects targeting bighorn are summarized in Table 12. These projects

Table 12. Bighorn-proof fence. Summary of fencing costs and performances at precluding bighorn sheep movements

Fence/Year constructed	Deploying party/ Funder	Fence dimensions (height x distance)	Materials	Landscape	Cost/km in USD in 2020	Estimated animal-years of exposure/# crossings
East Kootenay / Okanagan (2005; Zehnder 2006)		2.6m x 15km	High-tensile ("elk fence") game wire + inner domestic sheep page-wire fence		$310,000 over 15 km in 2005	
Red Rock Wildlife Area (Mooring et al. 2004)	USFWS (??)	2.6m X ??	Woven game fence			
National Bison Range (Unsworth JWM 1991)	USFWS	2.5m X ~25 miles	Woven wire fence	Palouse prairie		At least 2 in ~50 animal-years from 2018-2019
US 97A (north of Wenatchee WA)(Kelly McAllister abstract 2013)	WSDOT (WDFW to maintain)	2.4m X 9 miles	Woven wire fence	Rough, rocky, steep country	$2.8 million	18 sheep fatalities since fence construction in 2009 (but had had up to 9/yr without fence)
Antelope Island (south end; 2019)	UDWR	8 ft x 10.5 miles	6x6in mesh	99% mud flats, 1% flat grassland	$443,520	Currently unknown (but migratory deer are tracking along the fence and going around the ends)
Nevada highway fencing project: Wendover to Wells	NDOT/ NDOW	2.5m / 60 miles (associated with nine wildlife crossing structures)		Varies, but mostly flat		
Hypothetical	DOT-FHWA 2011	2.4m with post separation every 14-18 ft (4.2-5.4m)				
Hypothetical	Huijser et al. 2015	3.0m	Woven wire (12.5 gauge, mesh size 15-18 cm); wood posts 13cm in diameter for line posts, and at least 16cm for corners. No overhang required			
Hardware Ranch captive facilities	UDWR	10 ft	Woven wire, bars, black plastic (so not see-through)	Alpine meadow @ 5,500 ft.		1 for 15 ewes over 4 weeks (but jumping was instigated by capture pressure, and animals have not tested fence in other contexts))

reflect substantial underlying investment on the part of local agencies, and their efficacies at completely eliminating movement are somewhat equivocal.

Double-exclusion fences

Double-exclusion fences, or external fencing with internal pens, have the potential to both limit bighorn movement, and also provide for separation associated with direct contact (Zehnder 2006). Several designs, including a triangular suspended fence have been tested (Zehnder 2006); however, that design had a cost of $310,000 for 15 km in 2005. Heinse et al. (2016) suggest that paired fences within 2 meters of each other may provide a visual detractant to jumping. Since *M. ovipneumoniae* has been documented to transmit across distances greater than 2 meters (Brandi Felts personal communication), that distance may not be feasible. Furthermore, cost is likely prohibitive here: in Heinse et al.'s study, while 84% of producers were interested in double-fencing, only 16% of producers actually utilized it.

Internal pens

Heinse et al. found that 42% of the producers they surveyed (n = 40) had internal pens that could house their animals in the event that a bighorn arrived on their premises, and an additional 56% were interested in installing similar internal pens. Internal pens have the advantage of being cheaper to construct, but also require that a person be present and attentive on the premises to move sheep into pens on the rare occasion in which a bighorn might visit the operation.

Cost-benefit analysis of fencing

Costs of fence construction and maintenance

Several pieces of information that are pertinent for constructing a cost-benefits analysis of fencing design are currently unavailable, but we provide a preliminary assessment here. Chicken wire is likely sufficient material for many smaller operations, and chicken wire fencing typically costs $4-7 USD per linear foot for a 10' fence (cost estimates include labor, but not gates). We use a median price here of $5.50. Researchers at Iowa State estimate an 8% annual maintenance cost for chicken wire fence (Edwards & Chamra, 2012), and anticipate a 10-20 year life-time for these fences (Yard Fence Guide 2019; we assume a 15-year life expectancy here). To estimate costs of double-fencing, we simply double the cost of single-fencing, however this may slightly overestimate true costs.

We estimate annual cost of fence as follows:

Annual single fencing cost = (Cost of fence construction)/fence's life-expectancy

+ 8% (Cost of fence construction)

*= (Fence length (ft) * 5.50) / fence's life-expectancy + 0.08 * (Fence length (ft) * 5.50).*

Annual double-fencing cost = Annual single-fencing cost x 2

See Table 13 for cost estimates corresponding to various operation sizes.

Table 13. Fencing costs.

Area to fence	Perimeter length	Estimated cost	Annual cost (single fence)	Annual cost (double fence)
50 ft x 50 ft	200 ft	5.5 x 200 = $1100 US	(1100/15 year life expectancy) + (0.08 * 1100) = $161.33	$322.66
1 square acre	832 ft ()	5.5 * 832 = $4,576 US	$671.15	$1,342.30
5 acres (square)	1,866 ft (568m)	5.5 * 1,866 = $10,263 US	$1,505.24	$3,010.48
1 km	3281 ft	5.5 * 3,281 = $18,045.50	$2,646.67	$5,293.35
1 mile	5,280 ft	5.5 * 5,280 =$29,080	$4,265.07	$8,530.14

Cost of unfenced properties near bighorn range

We take the cost of unfenced domestic sheep operations near bighorn range as being equivalent to the cost of *M. ovipneumoniae*-associated disease events in the local bighorn herd times the probability that local bighorns acquire *M. ovipneumoniae* from the operation in question. We break these costs down as follows:

> *Cost of unfenced focal operation = Cost of M. ovipneumoniae-associated disease event x Pr(spillover occurs at focal operation)*
>
> *Cost of M. ovipneumoniae-associated disease event = Cost in die-off year + Cost in persistence year x Expected years of persistence*
>
> *Pr(spillover occurs at focal operation) = Pr(bighorn forays this far) x bighorn herd size x (ratio of operation boundary length to total length of foray arena) x Pr(infection | bighorn reaches operation)*

Each term in these equations is broken out in depth below.

Pr(Spillover occurs at focal operation)

Probability animal forays a given distance

O'Brien et al. (2014) estimate that 14.1% of rams and 1.5% of females go on forays in summer, and 17.9% of rams and 5.6% of females go on forays in winter. The probabilities that foraying animals cross various distances are included in O'Brien et al. 2014. Here, we take ewe foray risk as negligible, and focus entirely on foray risk arising from rams.

Ratio of operation boundary length to foray arena

Not all forays of a given distance *k* will cross a domestic sheep operation that is distance *k* away from the herd's core herd home range. If we assume that the direction of a foray is chosen at random, then we would expect the proportion of forays of distance *k* that cross an operation at distance *k* to be equal to the ratio of the operation's perimeter to the foray arena, that is, the circumference of a polygon extending *k* units beyond the herd's core use area. For example, if an operation's perimeter is 1/100 of the foray arena's total circumference at distance *k*, we

would expect 1/100 of forays of length *k* to encounter the operation. We calculate those ratios using two different assumptions about core herd home range configurations: a "round" core herd area and a "linear" core herd area. Proportions of forays expected to cross operations of various sizes at various distances from the core herd home range boundary are shown in Figure 1 below.

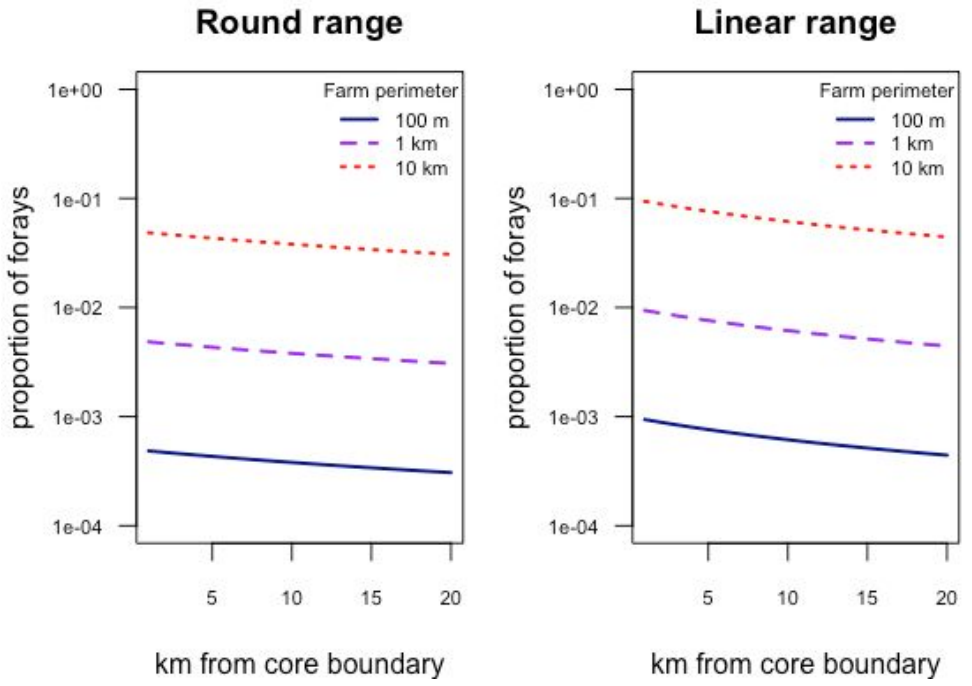

Figure 1. Proportion of forays expected to cross operations with specified perimeters (1km, 10km, 100km) at specified distances from the core herd home range boundary. Panels reflect two different extremes of core herd home range spatial configuration: Round (i.e., the occupied habitat is largely modular, so that diameter across the core herd home range is similar along any axis passing through the herd's center), and Linear (i.e., riverine systems in which occupied habitat extends much further along one axis through the system's center than along other axes).

Probability of infection given a foraying bighorn enters a domestic sheep operation

In a recent survey of U.S. domestic sheep operations, 88.5% of operations contained at least one animal that was *M. ovipneumoniae*-positive, and the median prevalence among positive operations was 60% (Manlove et al. 2019). If we assume that a foraying bighorn that enters a domestic sheep operation has 5 contacts appropriate for transmission, then the probability that it contacts at least one infected animal is one minus the probability that it encounters only uninfected animals. This latter probability of "escaping" *M. ovipneumoniae* encounter is $(1 - 0.60)^5$. The probability that the animal encounters at least one infected animal and becomes infected is one minus that escape probability = $(1 - ((1-0.60)^5))$. This quantity can then be rescaled according to the proportion of operations that harbored *M. ovipneumoniae* infection: 0.885 x Probability of becoming infected = $0.885 \times (1 - ((1-0.60)^5)) = 0.876$.

Cost of *M. ovipneumoniae* introduction events (USD throughout)

We partition *M. ovipneumoniae*-associated costs in bighorn herds into two categories: Costs in die-off years, and costs in post-die-off, persistence years.

Costs in die-off year

Costs in the die-off year are largely accrued through investigation efforts. We let these costs include 50 person-hours of evidence-gathering (collecting carcasses, performing necropsies, shipping diagnostic samples) = ~$2,500 under the assumption that labor costs average approximately $50/person-hour. We include an additional 50 person-hours in die-off management (decision making about management actions to take, along with implementation) = ~$2,500, again assuming an average cost of $50/person-hour. We include an additional $5,000 in operational costs (including vehicle mileage or helicopter use to gather samples, and diagnostic testing costs). These three components get us to a total cost of ~$10,000 in the year of the die-off.

Costs could be dramatically amplified depending on how much effort is invested in additional surveys (particularly when those efforts require helicopters), field follow-up efforts, and larger-scale diagnostic testing.

Cost in subsequent years (losses due to tags)

The most explicit cost associated with an *M. ovipneumoniae* introduction event is likely the cost associated with tag loss in subsequent years. Here, we assume a disease introduction event produces a 50% reduction in tag-associated income over the duration of the pathogen's persistence. Tag revenue can be compartmentalized into three broad categories: auction tag income, resident hunt income, and non-resident hunt income.

Here, we exclude on-going costs associated with recovering the herd, which regularly include more extensive capture, testing, collaring, and person-hours, along with sustained diagnostic testing costs. In cases where the pathogen is eradicated and herds are augmented, costs of augmentation (due both to capture and to acquisition of appropriate animals) may also be quite high. It is not unreasonable to think that herd recovery could exceed USD $100,000/year or more during an intensive recovery effort.

Auction tag income is typically on the order of $175,000 per province or state per year in recent years (this varies from state to state, and state-specific values should be applied directly here if known).

Resident hunt information can be applied as follows. In recent years, British Columbia has logged a median of 6,494 resident hunters across all 62 of its mountain sheep units from 2015 to 2018. Resident mountain sheep tags cost $60 in British Columbia (British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development 2020, pg. 10), and we assume the typical resident hunter spends ~$440 in additional costs (gasoline, equipment, etc.) for a total of $500 / resident hunter / year.

Non-resident mountain sheep hunters are required to hunt with a guide in British Columbia. Sportsmen's groups estimate a typical cost of $25,000 on a guided Rocky Mountain bighorn sheep hunt in BC (Meintzer, K. M. 2019), and BC has seen a median of 915.5 non-resident hunts across all mountain sheep units from 2015-2018.

Table 14. Economic benefit of fencing. Estimated economic benefits from a 90% reduction in risk of M. ovipneumoniae spillover associated with a fencing effort.

Distance from core herd home range	Probability a foraying animal goes this far	Ratio adjustment (assume 1km of operation boundary, and 100 km circumference of round bighorn core area)	Annual proportion of herd expected to foray this far	# animals expected to become infected (assuming bighorn herd has 25 males, with infection probabilities described below)	Waiting time to infection from this operation (years)	Expected proportion of years with disease (= persistence time/waiting time)	Expected cost (= pers to waiting * annual disease cost)	Annual benefit from fencing (assuming fencing reduces risk by 90%)
1 km	1	0.0048	0.0048 x 0.179 = 0.00087	0.00087 x 25 x 0.876 = 0.0190	52.62	0.21	$40,510.38	$36,459.34
5 km	0.80	0.0043	0.0043 x 0.179 = 0.00077	0.00077 x 25 x 0.876 = 0.0169	59.03	0.19	$36,110.78	$32,499.71
10 km	0.55	0.0038	0.0038 x 0.179 = 0.00068	0.00068 x 25 x 0.876 = 0.0149	67.05	0.16	$31,794.51	$28,615.06
20 km	0.23	0.0030	0.0030 x 0.179 = 0.00055	0.00055 x 25 x 0.876 = 0.0120	83.08	0.13	$25,660.24	$23,094.22
30 km	0.04	0.0026	0.0026 x 0.179 = 0.00046	0.00007 x 25 x 0.876 = 0.0101	99.10	0.11	$21,510.18	$19,359.16

Based on these values, total annual tag-associated income across all units is estimated as:

Auction tag value + resident hunters x *resident hunter expenditures + non-resident hunters* x *non-resident hunter expenditures*

*= 175,000 + 6,494 * 500 + 915.5 * 25,000 = $26,309,500*

If we split this across all 62 mountain sheep units in British Columbia, this produces an annual tag-associated income per unit of $424,346.80.

A 50% reduction in tag-associated income in years of disease persistence then corresponds to a $212,173.40 loss per persistence year, and this is the loss magnitude that we consider here. If we take the typical *M. ovipneumoniae*-associated disease event to continue affecting the bighorn population for 10 years of persistence (Cassirer et al. 2013; Manlove et al. 2016; Cassirer et al. 2018), then the total die-off cost can be estimated as

Cost of M. ovipneumoniae-*associated disease event = 10,000 + 424,346.80 * 0.5 * 10 (spread over 11 years) = $2,131,734.00*

with an annual cost = $2,131,734.00 / 11 = $193,794.00 for each of 11 years. The economic benefit from fencing is the reduction in this cost associated with installation of fence. Here, we assume that fencing reduces the probability of *M. ovipneumoniae* transmission from domestic to wild sheep by 90% reduction (though this reduction rate probability reflects reduction associated with double-fencing better than single-fencing, given the history of transmission following fenceline contact).

In the toy scenario above, the annual benefit of fencing (Table 14) always outweighs the annual cost of (double-) fencing a 1km area (Table 13, $5,293.35). Under the assumptions used here, the benefits always increase with increases in herd size.

Section VI Acknowledgements

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VII. Likely risk and consequences of *M. ovipneumoniae* transmission from domestic sheep and goats to wild mountain goats

M. ovipneumoniae infection status and consequences in mountain goats

M. ovipneumoniae's demographic consequences on mountain goats (*Oreanmos americanus*) have been studied in only a limited number of contexts, and thus there is more uncertainty about the consequences of *M. ovipneumoniae* infections in mountain goat populations than in bighorn sheep. That said, the evidence that does exist indicates that *M. ovipneumoniae* may sometimes have important effects on mountain goat population health.

A 2009-2010 *M. ovipneumoniae*-associated die-off event in two Nevada bighorn sheep populations (the East Humboldt and Ruby Mountains herds) was associated with some decline in the sympatric mountain goat population (Cox et al. 2017; Nevada Department of Wildlife unpublished data). The estimated decline in the goat population was substantially smaller than that in the bighorn population (estimated decline of 92% in local bighorns, but only 13% in the East Humboldt mountain goat herd, and 10% in the Ruby Mountains goat herd, though uncertainty in decline size for the goats likely exceeds the uncertainty in decline size for the bighorns). Follow-up studies on those mountain goat populations revealed that the goats were infected with the same *M. ovipneumoniae* strain that infected the local bighorns (Wolff et al. 2019), and that mountain goat kids exhibited poor summer survival, along with signs of respiratory disease (Blanchong et al. 2018) which was later confirmed to be *M. ovipneumoniae*-associated (Wolff et al. 2019). Aerial surveys and ground observation from 2011 to 2015 indicated decreased annual kid recruitment in both Ruby Mountains and East Humboldt Range goat herds (Cox 2017; Blanchong 2018), with summer kid survival estimates similar to those exhibited by bighorn herds infected with *M. ovipneumoniae* (summer kid survival estimated at 0.19 in the East Humboldt Range from 2013-2015; Blanchong et al. 2018).

Data from a variety of sources indicate that *M. ovipneumonia*e is present in a reasonable proportion of mountain goat herds, especially when those herds are sympatric with bighorn sheep populations (Table 15). Lowrey and coauthors (2018a) presented a survey of health status generated through the collaborative Greater Yellowstone Area Mountain Ungulate Project. They found *M. ovipneumoniae* circulating in both mountain goat populations that were sympatric with bighorn sheep herds, but did not detect *M. ovipneumoniae* in a goat population that did not overlap with bighorn sheep. However, this pattern of sympatry corresponding with *M. ovipneumoniae* infection did not extend to all herds. They found *M. ovipneumoniae* circulating in goat herds that were both sympatric and allopatric with bighorn sheep, and they did not detect evidence of *M. ovipneuoniae* circulation in herds that were both sympatric and allopatric with bighorn sheep. Several *M. ovipneumoniae* samples generated from mountain goats were included in the *M. ovipneumoniae* phylogenetic study of Kamath et al. 2019. All *M.*

Table 15. *M. ovipneumoniae* in mountain goats. Summary of *M. ovipneumoniae* status in mountain goat herds by population and year. "Shared strain" is shared *M. ovipneumoniae* strain, using current MLST methods.

Location (state)	Year	Lab	PCR (N/+/-)	Serology (N/+/-)	Sympatric with Bighorn / shared strain	Demographic consequences	Source
Northeast GYA (WY)	2013	WADDL/ WGF	14/3/10		Yes/		Lowrey et al. 2018a; demography from Smith and DeCesare
Northeast GYA	2014	WADDL	7/1/5	7/2/4	Yes/		Lowrey et al. 2018a
Southwest GYA	2013	WADDL	13/0/13	7/6/1	No/		Lowrey et al. 2018a
Southwest GYA (ID)	2014	WGF	9/9/0		No/		Lowrey et al. 2018a
Southwest GYA	2015	WGF	4/2/2		No/		Lowrey et al. 2018a
Southwest GYA	2017	WGF	4/2/1		No/		Lowrey et al. 2018a
Grand Teton NP (WY)	2014	WGF	5/0/5	5/0/5	Yes/		Lowrey et al. 2018a
Grand Teton NP (WY)	2015	WGF	4/0/4	4/0/4	Yes/		Lowrey et al. 2018a
Grand Teton NP (WY)	2017	WGF	5/0/5	4/0/4	Yes/		Lowrey et al. 2018a
Southeast Alaska	2010	WADDL	19/0/19		No/		Lowrey et al. 2018a
Southeast Alaska	2014	WADDL	14/0/14	16/0/16	No/		Lowrey et al. 2018a
East Humboldts (NV)	2010	WADDL	3/1/-	3/3/-	Yes/Yes	Estimated 13% decline in mtn goat pop size (Cox et al. 2017; NDOW data)	Wolff et al. 2019
East Humboldts (NV)	2012	WADDL	2/0/-	2/2/-	Yes/Yes		Wolff et al. 2019
East Humboldts (NV)	2013	WADDL	15/1/-	15/14/-	Yes/Yes	Summer kid survival estimated at 0.19 (Blanchong et al. 2018)	Wolff et al. 2019
East Humboldts (NV)	2014	WADDL	16/2/-	16/14/-	Yes/Yes	Summer kid survival estimated at 0.19 (Blanchong et al. 2018)	Wolff et al. 2019
East Humboldts (NV)	2015	WADDL	11/2/-	11/9/-	Yes/Yes	Summer kid survival estimated at 0.19 (Blanchong et al. 2018)	Wolff et al. 2019
Ruby Mtns (NV)	2012	WADDL	12/5/-	12/11/-	Yes/Yes		Wolff et al. 2019
Ruby Mtns (NV)	2013	WADDL	2/0/-	2/2/-	Yes/Yes		Wolff et al. 2019

Ruby Mtns (NV)	2014	WADDL	11/1/-	11/10/-	Yes/Yes		Wolff et al. 2019
Ruby Mtns (NV)	2015	WADDL	3/0/-	3/3/-	Yes/Yes		Wolff et al. 2019
Timpanagos (UT)	2017	WADDL	-/1/-		Yes/Yes	"The population has been fairly stagnant since 2011, but we think that they've had this strain for a long time, and the herd has grown at times while they've had this strain."	Kamath et al. 2019; Jace Taylor personal communication (02/27/2020)
Willard Peak (UT)	2015	WADDL	-/2/-		No/NA	"Willard grew quickly from 1994 to 2011 and has decreased almost as quickly since then. UDWR plans to collar goats in summer 2020 to monitor survival of adults and kids."	Kamath et al. 2019; Roug et al. 2017; Jace Taylor personal communication (02/27/2020)
Castle Creek (near Tom Miner) (MT)	2016	WADDL	-/1/-		??/Yes		Kamath et al. 2019
Battle Creek (SD)	2016	WADDL	-/1/-		Yes/Yes		Kamath et al. 2019

ovipneumoniae strains that were detected in mountain goats and included in Kamath et al. 2019 were classified as "domestic sheep" strains, as opposed to "domestic goat" strains (Kamath et al. 2019).

Several state wildlife agencies are currently prioritizing research into demographic consequences of *M. ovipneumoniae* infection in mountain goats (e.g., South Dakota Game, Fish and Parks, 2018); and *M. ovipneumoniae* is explicitly noted as a concern in other states (e.g., Idaho Department of Fish and Game 2019). However, current data on demographic trends in mountain goats commensurate with detection of *M. ovipneumoniae* remain sparse (consistent with information on all pathogens that mountain goats may harbor).

Risk of contact between mountain goats and other common hosts of *M. ovipneumoniae*

It is likely that the most extensive spatial overlap, and thus risk of pathogen transmission, between mountain goats and other hosts of *M. ovipneumoniae* is between mountain goats and bighorn sheep (Lowrey et al. 2018; Lowrey et al. 2018b), and this is especially true in systems where mountain goat range is expanding due to recent herd establishment (Gross 2001; Flesch et al. 2016). Shared resources (mineral licks, water, etc.) may be particularly important in facilitating bighorn sheep and mountain goat contact on the landscape, but remain understudied at this time.

In general, mountain goat terrain does not abut lands occupied by domestic sheep as extensively as does bighorn sheep terrain, though their strong patterns of seasonal vertical migrations (e.g., Rice 2008, White & Gregovich 2012) may occasionally bring mountain goats nearer to domestic sheep during winter. In one Alaska study, males had larger home ranges than females (White 2006), and substantial differences in movement patterns existed between the sexes during rut with males moving much more than females. In that study, female mountain goats had median seasonal home range sizes (based on 95% isopleths from a kernel density estimator with cross-validated smoothing parameters) of ~200-400ha each season. Males had home ranges of ~200 ha per season in fall and winter, but home ranges of around ~1200 ha during rut (White 2006). These are substantially smaller than reported home range areas for bighorn sheep calculated using similar methods (e.g., Oehler et al. 2003, who report 6,000-8,000ha home ranges for ewes in some desert bighorn herds; and Singer et al. 2001, who review published values for Rocky Mountain bighorns, and report values ranging from 6,000-35,000ha).

Summer space use patterns consist predominantly of animals moving deliberately from rocky outcropping to rocky outcropping. In one study, movement distances averaged ~60m per day, with some movements in excess of 6km (Shafer et al. 2011). In one Alaska study, males averaged 6,000m of movement over 5-day windows. Long moves could bring these animals into proximity with livestock, but their adherence to steep and rugged terrain may limit risk. However, mountain goats do sometimes use lower elevation mineral licks in the spring/summer (Hebert & Cowan 1971) which draw them nearer to domestic sheep and goats. Mountain goats are tightly tied to escape terrain, and one British Columbia study found declining use past 500m of escape terrain (Poole & Heard 2003).

Mountain goats exhibit landscape-scale genetic structure that suggests that some connectivity must exist at broader spatial scales, including between southern British Columbia and Washington state (Parks et al. 2015). The long-distance moves required to maintain genetic connection may occasionally bring mountain goats into higher risks of contacting M. ovipneumoniae carrier animals. Some risk of introduction may also exist when other hosts (for example pack goats) traverse through mountain goat terrain. However, the risk that either of these potential hosts would infect mountain goats in those brief passages is relatively low, due to both their own propensity for carrying *M. ovi* strains, as well as their concurrent animal husbandry practices (see Section V).

Section VII Acknowledgements

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VIII. Utility of *M. ovipneumoniae* strain typing, and variation in virulence of particular *M. ovipneumoniae* strains

The severity associated with particular *M. ovipneumoniae* introduction events in bighorn sheep seems to vary in the field (Cassirer et al. 2013; Manlove et al. 2016; Johnson et al., in prep), and *M. ovipneumoniae* strains occur at high diversity in domestic sheep (Kamath et al. 2019). A few well-studied cases suggest that some strains of *M. ovipneumoniae* may exhibit lower virulence than others (for example, strains originating in domestic goats, or strains originating from an apparently evolved clade in the Mojave desert, Kamath et al. 2019). To date, however, there has been no formal assessment of how extensively strain-to-strain variation shapes demographic responses following introduction in bighorn or domestic sheep, or in goats. A critical impediment to this assessment is the lack of an agreed-upon metric for "virulence". The Wild Sheep Working Group is currently considering three potential metrics: die-off size, pathogen persistence within a population, and induced serological titer produced by an exposure. All of these metrics are oriented towards understanding virulence in the wild host species, and none of them can be applied directly to inferring virulence in domestic hosts (and indeed, the high strain diversity exhibited by some domestic operations would likely mute any signal of virulence associated with particular strains).

Section VIII Acknowledgements

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