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

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ABSTRACT

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

1. Introduction

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

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

1.1. Background

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

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

Pathologic descriptions of bighorn sheep pneumonia were first provided in the first half of the 20th century (Rush, 1927; Marsh, 1938). Rush described a chronic pneumonia and noted lung adhesions, pus, "dark ulcers" and "a white strip around the edge of the lobes" in sheep necropsied in an all-age pneumonia outbreak that killed about 75% of the population on a portion of the Sun River game range in Montana in 1925. Marsh described a chronic pneumonia associated with lungworms (*Protostrongylus stilesi*), *Corynebacterium* (now *Trueperella*) *pyogenes* and *Pasteurella* spp. in winter pneumonia mortalities on the Sun River game range and Glacier and Yellowstone National Parks in the 1920s and 1930s, and an acute bacterial pneumonia associated with *Pasteurella* spp. and *T. pyogenes* in a high mortality summer pneumonia event affecting 2- to 3-month-old lambs at the National Bison Range. A similar variety of pathologies and agents continues to be observed in bighorn sheep pneumonia cases. More recently, additional lesions including rhinitis, otitis media, sinusitis, tracheitis, pleuritis, bronchiolar hyperplasia, and bronchiectasis have been associated with this disease (Cassirer and Sinclair, 2007; Besser et al., 2008). *M. ovipneumoniae* and respiratory viruses, especially parainfluenza-3 and respiratory syncytial virus, have also been added to list of potential pathogens (Aune et al., 1998; Weiser et al., 2003; Rudolph et al., 2007; Besser et al., 2008).

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

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

2. The role of lungworms

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

2.1. Strength of association

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

2.2. Plausibility

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

2.3. Experimental evidence

Experimental evidence is not supportive of the lungworm hypothesis. Experimental challenge of susceptible bighorn sheep with very high doses of infective lungworm larvae failed to produce any observed disease, refuting the possibility that bighorn sheep might be especially susceptible to lungworm induced pneumonia (Buechner, 1960; Forrester, 1971; Samson et al., 1987). Furthermore, anthelminthic treatments that effectively reduced lungworm burdens (Schmidt et al., 1979) were unsuccessful at improving lamb survival or preventing epizootics (Risenhoover et al., 1988; Goldstein et al., 2005).

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

3. The role of Pasteurellaceae

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

3.1. Strength of association

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

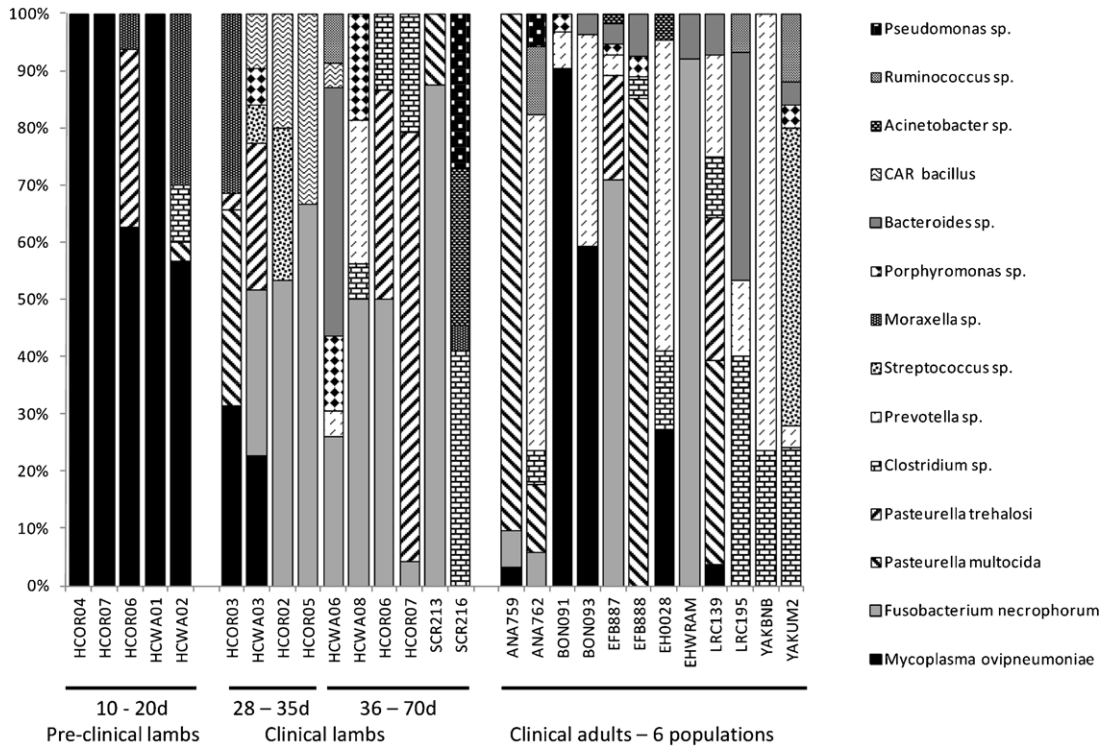

Fig. 1. Principal bacterial components of pneumonic bighorn sheep lung tissues based on 16S rDNA clone library analysis (Besser et al., 2008, 2012b). The *X*-axis legend specifies populations of origin of the bighorn sheep from which the tissues were sampled: HCOR = Hell's Canyon OR; HCWA = Hell's Canyon,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

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

3.2. Plausibility

As well established respiratory pathogens in many other animal hosts, Pasteurellaceae are generally plausible as causal agents of pneumonia in bighorn sheep. They also exhibit characteristics consistent with the long established association of bighorn sheep pneumonia with domestic sheep contact: bighorn sheep predominantly carry leukotoxin negative *B. trehalosi*, whereas most domestic sheep carry leukotoxin-expressing *M. haemolytica* (Sweeney et al., 1994; Jaworski et al., 1998). Therefore, the observed association of bighorn sheep pneumonia following domestic sheep contact is plausibly consistent with transmission of leukotoxin expressing Pasteurellaceae, particularly *M. haemolytica*. On the other hand, given the highly epizootic nature of this disease, a causal agent isolated from multiple affected animals within an outbreak would be expected to be indistinguishable by appropriate molecular epidemiologic 'fingerprinting' genetic tests. In a few cases, indistinguishable strain types (or strains carrying unique artificial markers) have been demonstrated in bighorn sheep experimentally commingled with domestic sheep or goats, consistent with transmission (Rudolph et al., 2003; Kelley et al., 2007; Lawrence et al., 2010). However, in general, indistinguishable (epizootic) Pasteurellaceae strain types have not been identified either within natural outbreaks (Weiser et al., 2003; Besser et al., 2012b) or in outbreaks following experimental commingling of domestic sheep and bighorn sheep (Onderka and Wishart, 1988; Foreyt, 1990, 1994; Foreyt, 1998), calling into question Pasteurellaceae as epizootic disease agents.

3.3. Experimental evidence

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

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

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

4. The role of *M. ovipneumoniae*

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

4.1. Strength of association

M. ovipneumoniae is strongly associated with pneumonia in bighorn sheep. The strength of association of *M. ovipneumoniae* was first investigated in a study of bighorn lambs collected in Hells Canyon (Besser et al., 2008). In addition to documenting the presence of *M. ovipneumoniae* in all pneumonic lambs but not in a healthy lamb, the study also included a serosurvey of nine additional pneumonic bighorn sheep populations, all of which exhibited high seroprevalence, and nine healthy bighorn sheep populations, all of which were seronegative. Subsequently, *M. ovipneumoniae* was detected in pneumonic lung tissues of >95% of 44 affected bighorn sheep lungs sampled in eight pneumonia epizootics that occurred in the western US during 2009–2010 but was absent in lung tissues of animals ($N = 5$) obtained from two populations unaffected by pneumonia (Besser et al., 2012b). Table 1 lists evidence of *M. ovipneumoniae* presence as tested by culture, PCR, or specific serologic antibodies in bighorn sheep populations of known health status from across western North America. Exposure to *M. ovipneumoniae* was documented in all 36 populations tested that were classified as pneumonic by the wildlife officials submitting the diagnostic specimens, but in only 3 of 32 populations that were classified as healthy ($P < 0.001$; odds ratio ∞/undefined; 95% confidence interval = $31 - \infty$).

4.2. Temporality

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

4.3. Plausibility

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

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

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

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

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

4.4. Experimental evidence

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

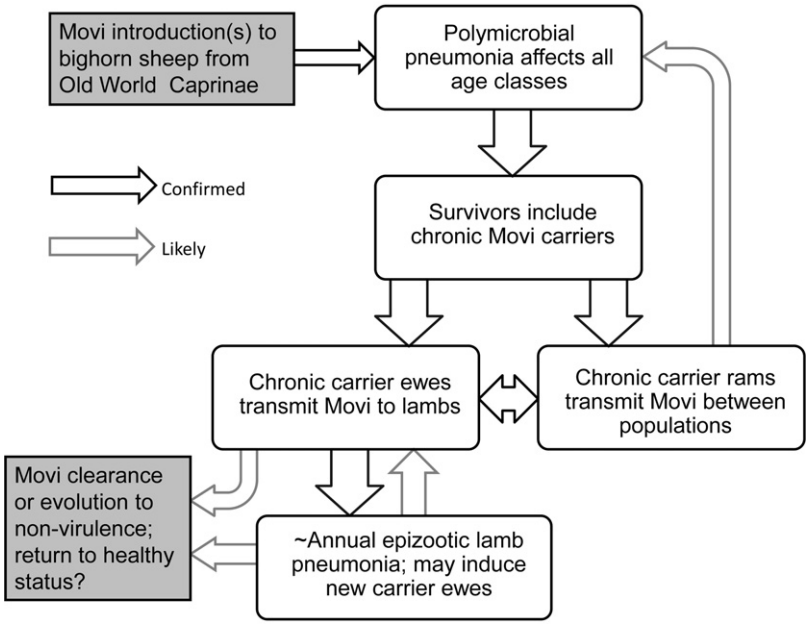

Fig. 2. Model diagram of the *M. ovipneumoniae* (Movi) hypothesis as presented in this paper.

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

4.5. Analogy

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

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

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

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

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

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

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