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Domestic sheep, bighorn sheep, and respiratory disease: a review of the experimental evidence

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Introduced infectious diseases pose a significant threat to wildlife populations and are exceptional conservation challenges, in part because they can precipitate much more rapid and devastating population declines than habitat encroachment. Pneumonia epizootics have played a major role in the dynamics and conservation challenges of bighorn sheep (*Ovis canadensis*) populations. A large proportion of native bighorn sheep populations south of Canada went extinct beginning in the second half of the 19th century. It has long been postulated, based on temporal and spatial correlations, that diseases transferred from domestic sheep (*Ovis aries*) played a major role in those losses. Although experimental research has repeatedly tested the hypothesis that domestic sheep carry strains of respiratory tract pathogens potentially fatal to bighorn sheep, debate continues over the role of domestic sheep in this disease process. In the context of a hierarchical set of hypotheses we review this experimental research that includes (1) contact trials involving bighorn sheep penned with domestic sheep and a variety of other native and domestic animal species; (2) inoculation experiments with no animal contact; (3) attempts to isolate and identify specific organisms responsible for pneumonia in bighorn sheep; and (4) vaccination experiments. Our review reveals that (1) experiments have repeatedly corroborated the hypothesis that bighorn sheep have a high probability of contracting fatal pneumonia following contact with domestic sheep; (2) low disease and mortality rates in numerous co-pasturing pen studies involving bighorn sheep and animals other than domestic sheep do not support the alternative explanation that the results of the co-pasturing studies involving domestic sheep were an artifact of captivity; (3) the identification of which organism(s) cause pneumonia in bighorn sheep following contact with domestic sheep remains unresolved, possibly because of disease complexity (multiple pathogens) and limitations of

research tools applied; and (4) vaccination trials largely have failed to mitigate the spread of respiratory disease and appear to be an unrealistic solution to the problem. We discuss these findings relative to a variety of questions, misinterpretations, and implications for management decisions concerning bighorn sheep conservation.

Key words: Bighorn sheep, domestic sheep, *Ovis aries*, *Ovis canadensis*, *Ovis dalli*, pneumonia, respiratory disease, *Pasteurella*, *Mannheimia*

Introduced infectious diseases pose a tremendous threat to wildlife. This threat increases as animal population sizes decrease, thereby reducing the gene pool of potentially resistant individuals, further increasing the likelihood of endangerment and extinction. Examples of the devastating effects of introduced diseases on wildlife are legion (Daszak et al. 2000), and the history of bighorn sheep (*Ovis canadensis*) provides a well-studied example that includes conservation actions taken to protect populations and reverse declining population trends through reintroductions. The tremendous amount of money and effort spent to repatriate bighorn sheep and the intense policy disputes over conservation strategies make this species an excellent case study of wildlife conservation in the face of disease. In this paper we review the scientific evidence for the most widely-cited hypothesis concerning the cause of many pneumonia epizootics in bighorn sheep — that bighorn sheep have a high probability of contracting fatal respiratory disease after contact with domestic sheep (*Ovis aries*), hereafter referred to as the "contact hypothesis". The implications of this hypothesis relative to bighorn sheep conservation and related decisions by governmental agencies that permit domestic sheep grazing on their lands has made this a contentious issue. Decision makers cannot be expected to study the diverse literature on this subject, yet have to make informed decisions in the face of pressure from both sides of this issue. A detailed review of this literature is clearly needed to help decision makers assess the scientific merit of various claims, as well as to synthesize existing information. Given that the introduction of domesticated animals has been connected with emerging infectious diseases in other wildlife (Daszak et al. 2000), the lessons learned from disease research on bighorn sheep also may have broader applications.

The original distribution of the two native sheep species in western North America, bighorn sheep and Dall's sheep (*O. dalli*), included suitable habitat north to the Brooks Range in Alaska, south to Baja California and the northern reaches of mainland Mexico, and east as far as west Texas and badland and river break habitats immediately east of the Rocky Mountains in North and South Dakota and western Nebraska (Buechner 1960, Valdez and Krausman 1999). In a large portion of this habitat in Alaska and Canada, the distribution of native sheep remains essentially unchanged (Valdez and Krausman 1999). In contrast, across much of the southern range of bighorn sheep, many populations were extirpated, including all native populations in the states of Washington, Oregon, and neighboring regions of southwestern Idaho, northeastern California, and northwestern Nevada (Buechner 1960). The states of California and Nevada together lost an estimated total of 110 native populations (McQuivey 1978, Wehausen et al. 1987). Restoration efforts were initiated during the 20th century to counter continuing population losses,

and by 1990 more than 8,000 bighorn sheep had been moved in 592 translocation efforts, primarily to restock vacant habitat (Ramey 1993).

The large region where bighorn sheep extirpations have been so widespread coincides spatially with where domestic sheep have been grazed in North America, and temporally with the beginning of that grazing. While one cannot infer cause and effect from spatial and temporal correlations alone, it has long been hypothesized that diseases transferred from domestic sheep were a key factor in the widespread loss of bighorn sheep populations. For example, the principal cause of the first large-scale population losses in the 19th century was attributed to scabies introduced by domestic sheep, based largely on clinical evidence of scabies in bighorn sheep during die-offs, and the temporal association of these scabies outbreaks with the introduction of domestic sheep (Honess and Frost 1942, Jones 1950, Smith 1954, Buechner 1960). Further negative correlations between the presence of domestic sheep and the health of bighorn sheep populations have emerged in the 20th century. In Nevada McQuivey (1978) noted a negative correlation between past domestic sheep grazing and the persistence of native bighorn sheep populations, and considerable circumstantial evidence has accumulated suggesting the hypothesis that die-offs of bighorn sheep frequently follow contact with domestic sheep (Goodson 1982, Martin et al. 1996, Singer et al. 2001, Coggins 2002, George et al. 2008). Where clinical evidence has been collected, pneumonia has been cited as the cause of death in those die-offs (Goodson 1982, Martin et al. 1996).

METHODS

During the past three decades various aspects of the potential role of domestic sheep in respiratory disease of bighorn sheep have been researched. Because scientific progress is limited in part by how problems are analyzed to formulate hypotheses and deduce testable (falsifiable) predictions, in this review of those research results we attempt to isolate separate questions and hypotheses concerning disease transmission between domestic and wild sheep. In so doing, we formulate a hierarchical series of hypotheses that are refinements of the contact hypothesis. We also approach this review from the standpoint of opportunities for hypothesis falsification. Popper (1959) identified falsifiability of hypotheses as the fundamental criterion of valid scientific inquiry of questions of cause and effect. He also argued that "proof" falls outside of the realm of science; instead, acceptance of hypotheses and the strength of such corroboration is a function of the attempts at and opportunities for falsification. We consider these concepts as fundamental to the disease questions that we review.

RESULTS

Our separation of this review into different questions and hypotheses lead us to partition the findings into six categories that facilitate the discussion of these different topics.

Unplanned pen experiments.—The contact hypothesis has been tested numerous times in captive situations. One set of tests has been accidental in nature and, therefore, lacked experimental design. However, the information garnered from those captivity situations still served as tests of the contact hypothesis. One unplanned experiment

occurred at Lava Beds National Monument, where in 1971 a population of bighorn sheep was established in a 5.4-km^2 enclosure (Blaisdell 1972). In 1980, nose-to-nose contact was observed through the enclosure fence between bighorn sheep and domestic sheep grazed on adjacent National Forest lands. Bighorn sheep began dying of pneumonia 2-3 weeks later and all 43 bighorn subsequently died (Foreyt and Jessup 1982). A second unplanned experiment involved bighorn sheep in Washington that had been in a 2.5-ha enclosure for 10 months when domestic sheep were added to the pen. Thirteen of 14 bighorn sheep subsequently died of pneumonia between 3 and 12 weeks after the introduction of the domestic sheep (Foreyt and Jessup 1982).

Planned pen experiments.—Following those unplanned experiments, 10 planned experiments specifically designed to test the contact hypothesis were carried out by three independent research groups using 1-6 captive bighorn sheep per trial. Four of those experiments used only domestic sheep (Onderka and Wishart 1988; Foreyt 1989, 1990, 1994), while contact in one (Foreyt 1994) involved mouflon sheep (*Ovis musimon*) and another five involved a mixed flock of domestic sheep and hybrids of argali (*Ovis ammon*) and mouflon sheep (Callan et al. 1991), the latter of which is the closest ancestor of domestic sheep (Ramey 2000, Hiendleder et al. 2002). The five trials involving hybrid sheep also included experimental treatments that attempted to control the resulting pneumonia in the bighorn sheep. Of the 23 bighorn sheep tested in those 10 trials, all died of respiratory disease following contact with domestic sheep, or were euthanized when close to death (Table 1). All domestic, mouflon, and hybrid sheep remained healthy.

Planned pen experiments with other species.—A couple of hypotheses might explain the planned pen results: (1) contact results in transmission of pathogens from domestic sheep to bighorn sheep that directly or indirectly lead to fatal pneumonia in the bighorn sheep (pathogen transmission hypothesis); or (2) the introduction of another species into the pen creates a negative psychological effect on the bighorn sheep, resulting in a compromised immune system leading to respiratory disease unrelated to the transmission of potential disease agents (stress hypothesis). Stress of behavioral origin similarly has frequently been hypothesized as an important factor in the livestock respiratory disease syndrome known as shipping fever (Hoerlein 1980, Yates 1982).

Table 1.—Summary of data from contact trials of bighorn sheep co-pastured with other species and inoculation trials of bighorn sheep and Dall's sheep (planned inoculation trials only) that used *M. haemolytica* cultured from domestic sheep.

	Number of trials	Wild sheep tested	Wild sheep dying	Domestic sheep and other species used	Domestic sheep and other species dying
Unplanned Pen Trials with domestic sheep	2	57	56		
Planned Pen Trials with domestic sheep	10	23	23	167	0
Planned Pen Trials with other species	9	55	4	32	0
Unplanned Inoculation Trials	1	13	6		
Planned Inoculation Trials	6	15	14	9	0

Nine independent contact experiments by Foreyt (1992a, 1994), Foreyt and Lagerquist (1996), and Foreyt et al. (2009) involving bighorn sheep penned with (1) elk (*Cervus elephus*), white-tailed deer (*Odocoileus virginianus*), and mule deer (*Odocoileus hemionus*); (2) elk alone; (3) domestic goats (*Capra hircus*); (4) mountain goats (*Oreamnos americanus*); (5) llamas (*Lama glama*); (6) cattle (*Bos taurus*); and (7) horses (*Equus caballus*) serve as a test of the stress hypothesis (Table 2). Of 55 bighorn sheep tested in those experiments, only four died (Table 1). One was an old female whose death most likely was due to a tooth anomaly that adversely affected her feeding ability. The other deaths were a bighorn sheep in the experiment with steers that died of pneumonia (Foreyt and Lagerquist 1996) and two of seven bighorn co-pastured in one trial with domestic goats that died of pneumonia caused by *Mannheimia haemolytica* (Angen et al. 1999) biotype A, serotype 2 (Foreyt et al. 2009; Table 2). The significantly ($P<0.001$; chi square test) lower proportion of bighorn sheep dying in pen trials that put bighorn sheep in contact with other species compared with experiments involving contact with sheep of Old World origin (Table 1) does not support the stress hypothesis. Instead, these findings suggest that the presence of other species in pens itself is unlikely to lead to bighorn sheep deaths and, furthermore, that species other than domestic sheep and their relatives are considerably less likely to transmit pathogens potentially fatal to bighorn sheep. This conclusion is consistent with a lack of circumstantial data linking most of these other species to bighorn sheep die-offs. Domestic goats appear to be the exception (Rudolph et al. 2003), and recent findings indicate that they also can carry other disease organisms with serious consequences for bighorn sheep (Jansen et al. 2006). However, the lack of disease transmission to bighorn sheep by the other species tested does not imply that they lack respiratory tract organisms pathogenic to bighorn sheep; instead, lack of disease may result from interspecific behavioral patterns that largely preclude contact and pathogen transmission.

Table 2.—Details from contact experiments involving bighorn sheep co-pastured with domestic sheep (Dom. sheep), mouflon sheep, domestic goats (Dom. goats), white-tailed deer (W-T deer), mule deer, elk, mountain goats (Mt. Goats), llamas, horses, and cattle.

		Co-pastured with:										
Bighorn used	Bighorn dead	Dom. sheep	Mouflon sheep	Dom. goats	W-T deer	Mule deer	Elk	Mt. goats	Llamas	Horses	Cattle	reference
2	2	2	-	-	-	-	-	-	-	-	-	Onderka and Wishart 1988
6	6	6	-	-	-	-	-	-	-	-	-	Foreyt 1989
2	2	2	-	-	-	-	-	-	-	-	-	Foreyt 1990
6	6	-	5	-	-	-	-	-	-	-	-	Foreyt 1994
2	2	2	-	-	-	-	-	-	-	-	-	Foreyt 1994
1	1	30[a]	-	-	-	-	-	-	-	-	-	Callan et al. 1991
1	1	30[a]	-	-	-	-	-	-	-	-	-	Callan et al. 1991
1	1	30[a]	-	-	-	-	-	-	-	-	-	Callan et al. 1991
1	1	30[a]	-	-	-	-	-	-	-	-	-	Callan et al. 1991
1	1	30[a]	-	-	-	-	-	-	-	-	-	Callan et al. 1991
10	0	-	-	-	2	1	4	-	-	-	-	Foreyt 1992a
3	0	-	-	-	-	-	4	-	-	-	-	Foreyt 1992a
9	0	-	-	-	-	-	-	2	-	-	-	Foreyt 1994
9	0	-	-	-	-	-	-	-	3	-	-	Foreyt 1994
4	0	-	-	-	-	-	-	-	-	-	3	Foreyt 1994
5	1	-	-	-	-	-	-	-	-	-	3	Foreyt and Lagerquist 1996
6	1[b]	-	-	-	-	-	-	-	-	3	-	Foreyt and Lagerquist 1996
2	0	-	-	3	-	-	-	-	-	-	-	Foreyt 1994
7	2	-	-	4	-	-	-	-	-	-	-	Foreyt et al. 2009

[a] mixture of domestic sheep and mouflon-argali hybrids
[b] animal in poor condition at beginning of experiment; death likely caused by tooth anomalies and feeding difficulty.

Inoculation experiments.—The pathogen transmission hypothesis can be further refined to the fatal strains hypothesis (Goodson 1982): that specific species, microbial strains, or viruses frequently carried by healthy domestic sheep are the cause of fatal pneumonia in bighorn sheep following contact between these species. This hypothesis has been tested by experiments in which captive bighorn sheep have been inoculated with bacteria cultured from the respiratory tracts of domestic sheep. Similar to the contact experiments, this has involved both accidental and planned experiments. The accidental experiment occurred when a lavage tube used to sample lung cells of domestic sheep was not fully sterilized before being used to obtain lung cultures from three captive bighorn sheep. All 10 bighorn sheep in this herd developed pneumonia, of which three died, as did three additional bighorn sheep added to the herd (Foreyt 1990).

The planned inoculation experiments comprise six independent trials carried out by two different research groups using *M. haemolytica* cultured from domestic sheep (Onderka et al. 1988, Foreyt et al. 1994, Foreyt and Silflow 1996). Of 13 bighorn sheep that were inoculated with those bacteria, 12 died of acute bronchopneumonia. Two groups of control bighorn sheep (five total) remained healthy, as did two groups of domestic sheep (nine total) that received the same inoculation doses as the bighorn sheep (Table 1). Two of these inoculation trials (Onderka et al. 1988, Foreyt and Silflow 1996) also included experiments in which the source of the *M. haemolytica* inoculum was cultured from healthy bighorn sheep. The three bighorn sheep used in those two trials showed no clinical signs of disease after the inoculations, and neither did seven domestic sheep similarly inoculated.

Foreyt et al. (1996) also carried out an inoculation trial of three Dall's sheep (*Ovis dalli dalli*). Two of these sheep received a *M. haemolytica* strain (A2) from domestic sheep that by inoculation trials was fatal to bighorn sheep, while the other received a strain not considered to be pathogenic. The sheep receiving the non-pathogenic strain remained healthy; the other two developed bronchopneumonia, from which one died, and one was euthanized prior to death.

Dassanayake et al. (2009) used 10 bighorn and 12 domestic sheep to test two forms of the *M. haemolytica* A1 strain in inoculation trials. Two bighorn and two domestic sheep were controls, while four of each species received the wild type A1 strain, and the other four received a mutant A1 form that lacked the leukotoxin gene (Murphy et al. 1995). One control domestic sheep died of causes unrelated to the experimental treatment. All other sheep survived without clinical pneumonia except the four bighorn sheep that received the wild type A1 strain, all of which died of acute bilateral pneumonia within 48 hours. These results appear to expand the list of strains fatal to bighorn sheep. However, the *M. haemolytica* A1 strain used was identified only as wild type with no information on its source (Dassanayake et al. 2009, Murphy et al. 1995).

Besser et al. (2008) tested the role of *Mycoplasma ovipneumoniae* alone in this disease process by inoculating two young bighorn lambs. Neither showed signs of clinical pneumonia.

Research to identify bacterial strains causing fatal pneumonia in bighorn sheep.—The results of the various contact and inoculation trials corroborate the pathogen transmission and fatal strains hypotheses. With sufficient diagnostic tools, it should theoretically be possible to identify the specific strain(s) of bacteria or other pathogens that cause fatal pneumonia in bighorn sheep. However, the goal of identifying all specific pathogens has proven elusive. Multiple bacterial species have been implicated as causing

disease in bighorn sheep. While *M. haemolytica* has been cultured from many bighorn sheep dying of pneumonia following experimental contact with domestic sheep, especially the A2 strain, one set of experiments attributed the deaths instead to *Pasteurella multocida* (Callan et al. 1991). Additionally, some forms of *M. haemolytica* are now recognized as a separate species, *P. trehalosi* (Sneath and Stevens 1990). Traditional methods used to differentiate strains of *M. haemolytica* by biotypes and serotypes (Dunbar et al. 1990a, 1990b; Queen et al. 1994) have lacked adequate resolution. Previously unknown serotypes have been found in bighorn sheep (Dunbar et al. 1990a), while other strains could not be identified using these methods (Dunbar et al. 1990a, Silflow et al. 1994, Sweeney et al. 1994, Ward et al. 1997), rendering these classification methods unsatisfactory for epidemiological investigations of this phenomenon (Jaworski et al. 1993).

To overcome limitations of traditional methods, additional diagnostic tools have been applied to *M. haemolytica* and *P. trehalosi* in attempts to develop more refined classifications that might better identify strains responsible for bighorn sheep deaths. These measures have included (1) binary classification as hemolytic or non-hemolytic (Wild and Miller 1991, 1994; Ward et al. 2002); (2) variation in surface proteins (Ward et al. 1990); (3) assays of toxicity relative to peripheral neutrophils (Silflow and Foreyt 1994, Silflow et al. 1994, Sweeney et al. 1994); (4) DNA fingerprinting to identify different genetic forms (Snipes et al. 1992, Jaworski et al. 1993; Foreyt et al. 1994, Ward et al. 1997, Weiser et al. 2003); and, (5) culture-independent PCR-based methods and sequence-based phylogenetic analyses of multiple genetic loci (Safaee et al. 2006, Kelley et al. 2006, Besser et al. 2008).

Silflow et al. (1989) found no differences between bighorn sheep and domestic sheep in a number of immune system measures involving phagocytes. In contrast, Silflow et al. (1993) identified a mechanism involving lysis of neutrophils by a cytotoxin produced by some *M. haemolytica* strains that might explain the high susceptibility of bighorn sheep to specific strains of *M. haemolytica*. Comparisons of neutrophil sensitivity to this cytotoxin for five native North American ungulates and domestic sheep found bighorn sheep, and especially Dall's sheep, to be notably more susceptible to neutrophil destruction than the other species tested (Silflow and Foreyt 1994, Silflow et al. 1994). While strains of *M. haemolytica* fatal to bighorn and Dall's sheep consistently showed high toxicity in cytotoxicity assays (Foreyt and Silflow 1996, Foreyt et al. 1996), other cytotoxic strains have not caused significant respiratory disease in bighorn sheep (Foreyt and Silflow 1996); thus, this cytotoxicity classification alone lacks adequate predictive power relative to respiratory disease in bighorn sheep. The same can be said of the other diagnostic methods. While DNA fingerprinting has been useful for investigating transmission of bacterial strains between different species and individuals (Ward et al. 1997), these methods also appear to lack predictive power relative to identifying strains that can cause fatal pneumonia in bighorn sheep.

One possible explanation for the failure of these diagnostic methods to consistently identify bacterial strains fatal to bighorn sheep is that the culturing methods they depend on do not identify most members of the microbial community sampled. Results from culture-independent PCR-based methods indicate that culture-based methods typically miss about 99% of microbial diversity in any given biological sample (Amann et al. 1995, Hugenholtz and Pace 1996, Tanner et al. 1999, Eckburg et al. 2005), including sheep respiratory tracts (Safaee et al. 2006, Besser et al. 2008). Furthermore, several studies have found evidence that horizontal gene transfer of the leukotoxin gene has occurred

among *Mannheimia/Pasteurella* species sampled from different species and locales in both domestic (Davies et al. 2001, Davies et al. 2002) and wild sheep populations (Kelley et al. 2006). This same mechanism contributes to virulence in other bacteria, including shiga toxin, cholera toxin, and neurotoxins of *Clostridium botulinim* (Novick 2003). While Kelley et al. (2006) found that DNA sequences from *Mannheimia* and *Pasteurella* obtained from different host species and locales tend to form closely related clusters, horizontal gene transfer of leukotoxin and other virulence genes may explain a lack of correspondence between strains identified using traditional methods and their virulence. Evidence of extensive recombination of the toxin genes within *P. trehalosi and M. haemolytica* (Davies et al. 2001) suggests that presence of this gene in a population of *Mannheimia* or *Pasteurella* does not necessarily mean that it is virulent. Most recently, using culture-independent approaches, Besser et al. (2008) found evidence suggesting involvement of *Mycoplasma ovipneumoniae* in bighorn sheep respiratory disease.

Vaccination trials.—Vaccination of wild animals is logistically difficult at best in most situations and even more so for bighorn sheep because of the steep, craggy, relatively inaccessible habitat they often inhabit. Additionally, some vaccines require multiple doses to stimulate initial immune system response. Thus, vaccination is not a viable disease management option for most wild populations. Nevertheless, vaccination experiments have been carried out and might have applications to captive wild sheep and occasional free-ranging situations.

Ward et al. (1999) investigated immunologic responses of bighorn and domestic sheep to a vaccine against three strains of *M. haemolytica*. They found that the vaccine produced only a moderate and transient immunologic response. Miller et al. (1997) and Kraabel et al. (1998) tested a vaccine for three different *M. haemolytica* strains on captive bighorn sheep. The sheep were challenged with *P. trehalosi* cultured from lungs of free-ranging bighorn sheep during a pasteurellosis epizootic. Control and vaccinated bighorn both developed acute pneumonia, but vaccinated ones experienced lower mortality (30% vs. 80%).

For multiple years following pneumonia epizootics in bighorn sheep, it is common for most lambs of surviving females to die of pneumonia (Foreyt 1990, Coggins and Matthews 1992). Cassirer et al. (2001) conducted experiments with free-ranging and captive bighorn to test the efficacy of vaccines against *Mannheimia/Pasteurella* to reduce such lamb mortality, but vaccinated females had notably higher loss of lambs than non-vaccinated ewes.

Only two vaccination trials have used strains of *M. haemolytica* derived from domestic sheep as the post-vaccination challenge. Foreyt and Silflow (1996) inoculated two bighorn sheep twice with a non-lethal cytotoxic strain of *M. haemolytica* and six weeks later inoculated them with a lethal cytotoxic strain (A2) from domestic sheep. The non-lethal strain provided no significant protection, and both bighorn sheep died of bronchopneumonia. Foreyt (1992b) tested an experimental bacterin-toxoid vaccine for three *M. haemolytica* strains, using three treatment and three control bighorn sheep. After contact with domestic sheep, five of the six bighorn sheep, including the three vaccinated ones, died of pneumonia, with no evidence of any protection from the vaccine.

Discussion

A variety of field observations spanning many decades led to the hypothesis that bighorn sheep have a high probability of developing fatal pneumonia following contact with domestic sheep. Subsequently, numerous independent experiments have tested this contact hypothesis, and the results have repeatedly corroborated it (Table 1, Table 2). There have been numerous opportunities to falsify the contact hypothesis under controlled conditions and none has done so. Many bighorn sheep have died in those experiments, and it seems unlikely that more such experiments will add further knowledge to the contact hypothesis.

The stress and pathogen transmission hypotheses were proposed as two basic mechanisms to explain the results of contact experiments; but only the pathogen transmission hypothesis was consistent with the experimental data. The pathogen transmission hypothesis and the more refined fatal strains hypothesis have been tested and corroborated by *M. haemolytica* inoculation experiments. While the realistic nature of the inoculation doses might be questioned, domestic sheep similarly inoculated remained healthy, as did control bighorn sheep; and similar inoculation doses of *M. haemolytica* strains cultured from bighorn sheep produced no clinical effects in either sheep species. These results are consistent with expectations from the fatal strains hypothesis and provided opportunities for falsification.

The effort to identify organisms causing pneumonia in bighorn sheep following contact with domestic sheep has not yielded simple answers; instead, this situation appears complex with many potentially pathogenic bacteria of multiple species identified. In part, this may reflect limitations of the technology applied to this question. New culture-independent methods are greatly expanding knowledge of microbial communities inhabiting animals (Eckburg et al. 2005) and are beginning to shed new light on disease transmission (Tanner et al. 1999). However, the hunt to identify organisms causing pneumonia in bighorn sheep appears to have been limited by a traditional search for specific bacterial species or strains. The expanding understanding of potential mechanisms underlying pathogenicity in other diseases, e.g., horizontal gene transfer (Schubert et al. 2009), may explain why such a traditional approach has not been successful for bighorn sheep. Indeed, even criteria for defining bacterial species remain unclear (Fraser et al. 2009).

Definitive identification of pathogens causing fatal pneumonia in bighorn sheep is a question of scientific interest that may ultimately have practical applications. However, the inability to definitively and consistently identify pathogens responsible for all bighorn sheep deaths following contact with domestic sheep does not have bearing on the question of whether such contact has a high probability of leading to deaths of bighorn sheep. These are different questions that frequently have been inappropriately intertwined. Shipping fever is a similar respiratory disease problem that costs the U. S. livestock industry many millions of dollars annually (Rehmtulla and Thomson 1981); yet, it also has not yielded a single causative disease agent despites decades of intensive research (Storz et al. 2000).

A glance backwards to the early days of human public health shows that stalling epidemics has not required complete knowledge of the disease mechanism or identification of the pathogen. Without any knowledge of the microbial cause of cholera, John Snow hypothesized that the source of the 1849 London epidemic as water from one well. He

tested his hypothesis by removing the handle to the pump for that well, which provided corroboration when the epidemic ended abruptly (Glass 1986, Garrett 1994). His hypothesis was analogous to our contact hypothesis and his scientific conclusions did not require knowledge of the specific pathogen causing the disease. In fact, it was another three decades after Snow halted that particular epidemic before the cholera bacterium was established as the cause of that disease (Howard-Jones 1984). Because city planners refused to accept Snow's reasoning that water contaminated by sewage was the source of the epidemic, cholera outbreaks continued to plague London for decades (Garrett 1994). The resistance of some to the apparent role of domestic sheep in bighorn sheep pneumonia suggests a parallel situation.

One of the principal reasons some critics have cited for doubting the contact hypothesis is that Koch's postulates for establishing a causative relationship between a microbe and a disease have not been convincingly fulfilled. Among other things, Koch's postulates propose that to identify a microbial agent as the cause of a human disease, it is necessary to isolate the same organism from each case of the disease, and to produce that disease in an animal by inoculating it with that agent cultured from a diseased individual (Fredericks and Relman 1996). While the same postulates apply to animal diseases, Hanson (1988) concluded that the application of Koch's postulates to the study of wildlife diseases was a simplistic approach to a complex situation that had little meaning given current knowledge and technology, and this general concern has been echoed by others (Evans 1976, Fredericks and Relman 1996). Indeed, Koch himself later recognized that his postulates could not be satisfied in every case (Fredericks and Relman 1996). The respiratory disease relationship between domestic and bighorn sheep appears to epitomize that conclusion. By the definition of a disease implied by Koch's postulates, the disease phenomenon reviewed here may involve multiple disease processes involving multiple microbial species and strains. Additionally, a lesson from studies using culture-independent PCR methods is that Koch's postulates can be applied to only a small fraction of potential pathogens that can be cultured for inoculation.

This review examined only the experimental evidence concerning whether domestic sheep are a likely source of respiratory pathogens potentially fatal to bighorn sheep. How any situation of potential contact between these species in the wild will play out is a complex question that involves a series of probabilistic events. First is the probability of contact between the two species. Second is the probability that pathogenic strains are transferred. Third is the probability that pathogen transmission will lead to pneumonia, a probability possibly influenced by the status of the immune system of the bighorn(s) receiving pathogenic strains relative to the dose received. Fourth is the process of pathogen transfer within an infected bighorn sheep population. Fifth is the probability of death of infected individuals, which will likely vary among populations due to multiple variables, including genetic constitution of the herd, nutrition, environmental stressors, and the virulence of pathogen(s). Because post die-off population dynamics are often influenced by survivors of such pneumonia epizootics that carry and transmit respiratory tract pathogens to lambs for years (Foreyt 1990, Coggins and Matthews 1992), there are questions of yet longer term interactions between herd immunity and pathogens. Below we touch on a few questions of this larger disease question.

Sheep in general are susceptible to pneumonia, and bighorn sheep appear particularly susceptible to this disease, exhibiting periodic pneumonia die-offs in the Rocky Mountain region (Buechner 1960, Stelfox 1971). While some of these epizootics can be traced to

contact with domestic sheep and subsequent inter-population migration of pathogens within metapopulations (Goodson 1982, Onderka and Wishart 1984, George et al. 2008), there is a large literature that we do not review documenting pneumonia outbreaks and die-offs in bighorn sheep populations with no known recent prior contact with domestic sheep (Goodson 1982, Martin et al. 1996). Researchers typically have attributed these latter pneumonia outbreaks to various environmental conditions likely to predispose wild sheep to respiratory disease (Festa-Bianchet 1988, Monello et al. 2001), but Hobbs and Miller (1992) suggested that such conditions might not be necessary. However, the lack of any documented pneumonia epizootics in the large expanse of wild sheep range in Canada and Alaska, where there has been almost (Heimer et al. 1992) no opportunity for direct or indirect contact with domestic sheep (Hoefs and Cowan 1979, Hoefs and Bayer 1984, Monello et al. 2001, Jenkins et al. 2007) is a pattern needing explanation. Among potential hypotheses is that bighorn sheep populations that have survived past pneumonia epizootics resulting from contact with domestic sheep continue to carry respiratory microbes from domestic sheep that (1) are lacking in Alaska and most of Canada; and, (2) render these bighorn sheep more susceptible to pneumonia when various environmental conditions converge to compromise immune systems and/or there is an evolutionary change in pathogen virulence.

The role of predisposing factors in outcomes of pneumonia epizootics of wild bighorn sheep populations stemming from recent contact with domestic sheep also is unclear. Results from pen experiments suggest that the virulence of pathogens transferred in such contact can overpower the immune system of bighorn sheep regardless of prior physical condition and diet quality; but, the applicability of experimental results to wild situations has nevertheless been questioned, and such epizootics in the wild do vary considerably in the proportion of the herd that dies (ca. 50-100%; Goodson 1982, Martin et al. 1996). While extensive replicated experiments on wild populations would be desirable to help clarify cause and effect, it is doubtful that such research will occur. Statistically it would be appropriate to have at least three treatment and three control populations. Given the value of the bighorn sheep resource and the implications of the existing data reviewed here, it is unlikely that any agency with jurisdiction over bighorn sheep would be willing to subject multiple healthy populations of bighorn sheep to the risk of a severe pneumonia epizootic resulting from such an experiment. Ethical questions also might arise. As scientific experiments, the pen trials we reviewed were carried out specifically to control as many confounding variables operating in wild populations as possible in order to best measure the effects of the variable of interest. In that regard those pen trials potentially yield more important information than might be obtained from experiments involving wild populations. Contact between domestic and bighorn sheep in the wild may not always produce the same consistency of results seen in controlled pen studies because of variables outlined above; however, it is well known in epidemiology that probabilities of disease transmission to susceptible hosts increase with repeated exposure (Frerichs 1995). Consequently, greater variation in observations from wild situations might be expected relative to results from pen studies. A prediction from the results of pen studies reviewed here might be that repeated opportunities for contact between domestic sheep and bighorn sheep eventually will lead to a pneumonia epizootic in the bighorn sheep. Aune et al. (1998) documented this for one bighorn sheep population in Montana.

Relative to resource management decisions, the pertinent question is whether bighorn sheep have a high probability of developing fatal pneumonia following contact with domestic sheep. While desirable, it is not necessary to completely understand details of the disease process, or even identify responsible pathogens, to make appropriate management decisions. Relative to other judgments that must be made by resource management agencies, the potential effect on bighorn sheep of contact with domestic sheep appears remarkably clear cut. Where the health of any bighorn sheep populations is valued, the recommendation has been management actions that prevent contact with domestic sheep (Foreyt 1994, Foreyt et al. 1994). Such contact can occur in two ways: stray domestic sheep contacting bighorn sheep, or bighorn sheep contacting domestic sheep bands and spreading pathogenic microbes to other bighorn sheep. Keeping an adequate spatial buffer between bighorn sheep and domestic sheep has been considered the most reliable method to prevent contact between these species (Desert Bighorn Council Technical Staff 1990, Bureau of Land Management 1992, Schommer and Woolever 2001, Singer et al. 2001). However, this solution may not always be adequate because of distances bighorn sheep males sometimes travel, and politically is seldom simple to achieve. Depending on the situation, other approaches may be possible. Finding a management solution to this problem is dependent on the parties first agreeing that contact between domestic and bighorn sheep is a significant health threat for bighorn sheep. It is our hope that this review will help assure that such agreements will be based on a complete and critical review of pertinent scientific information that separates different falsifiable hypotheses, and thereby does not mix questions that should be addressed independently.

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