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Respiratory Disease in Mountain Sheep: Knowledge Gaps and Future Research

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Workshop Day 1

Welcome, Introductions, and Workshop Overview¹

Dr. Ben Gonzales, California Department of Fish and Game (CDFG), welcomed participants and introduced the organizing committee: Dr. Ben Gonzales; Dr. Frances Cassirer, Idaho Department of Fish and Game; Dr. Elena Garde, Sitka Veterinary Services; Dr. Dave Jessup, California Department of Fish and Game.

Melanie Woolever, U.S. Department of Agriculture, provided opening remarks, speaking about the Forest Service's interest in bighorn sheep. Approximately 95% of bighorn sheep live on National Forest lands; what happens on those lands is important to their survival. The Forest Service must use the best-available science to provide for species persistence in a politically charged environment. Biologists must have solid scientific information when making resource management recommendations. Woolever admitted that science may not solve politically based issues, but there must be a science-based foundation when discussing the proper use of resources. She asked participants to step back from their focused area of expertise for the next two days and to look for opportunities for collaboration. She sees a lot of duplicate projects submitted for funding. Researchers need to be strategic when asking for funds and they need to remember that the National Forest provides for multiple uses. The Forest Service is doing their best to ensure a fair balance in use and to ensure that bighorn sheep thrive in National Forest lands. However, the Forest Service can't succeed alone and she urged participants to be part of the solution. She reminded participants that progress made at this workshop will address the future of bighorn sheep. Woolever asked everyone to share his or her talents, information, and even funding. In closing, she expressed her excitement and hopes that each participant would put competition aside and work together to find solutions.

Ray Lee, Foundation for North American Wild Sheep (FNAWS), also provided welcoming remarks as a workshop sponsor. FNAWS has spent a lot of money to "put sheep on the mountain," but have noticed it is difficult to keep them there. Lee has observed the many ways that sheep have been dying and has seen herd numbers fall dramatically after interacting with domestic sheep. Although responsibility has not been assigned for these large die-offs in the past, Lee has seen a change. He cited a recent case in Arizona where disease in a wild sheep herd was attributed to domestic goats, and both criminal and civil lawsuits were filed against the domestic goat owner. FNAWS has worked with both domestic sheep landowners and government agencies to manage domestic sheep in bighorn sheep habitat. Efforts by FNAWS have included promoting a change in livestock from sheep to cattle and providing financial compensation to 401K programs for sheepherders, which can be expensive and has become increasingly complicated. Members of FNAWS realize that funding research is a better use of resources. FNAWS does not intend to force domestic sheep off of the national forests, but wants to make the public aware that not every acre can be used for multiple uses: some areas of the national forest should not support domestic sheep.

¹ The meeting summary is arranged chronologically: presentations from day one are followed by the corresponding question and answer sessions and working group presentations from day two complete the document. The meeting agenda is located in Appendix A and a complete list of participants is presented in Appendix B. Summaries from each working group are organized in Appendices C through F and copies of working group flip charts are provided in Appendix G.

After trying to follow the adoption of management plans by the Bureau of Land Management and the Forest Service, Lee realized that all parties involved need to be on the same page if government leaders are going to enact change. A recent example cited was the Payette National Forest. FNAWS wants to fund the most promising scientific research, especially collaborative projects. Lee acknowledged people from all sides of the issue at the meeting and stated that he was very proud of the opportunity to attend today.

Dr. Frances Cassirer provided welcoming remarks on behalf of the organizing committee. She noted that the committee has over 40 years of combined experience working with bighorn sheep. Each member believes the issues facing bighorn sheep are complex and that a collaborative approach is needed to find solutions. The organizers know that everyone attending has special expertise and hopes that each participant will share that expertise, get new ideas, make connections with others, and participate in future collaborations. Dr. Cassirer added that the purpose of the workshop is to discuss the science related to respiratory disease in bighorn sheep and that scientific discourse and criticism are welcome. Finally, she acknowledged those who invested time and resources to make the workshop a reality.

Workshop Overview

Susan Hayman, North Country Resources, introduced herself and Nikole Pearson, Peak Science Communications, and explained their respective roles as facilitator and technical writer/editor. She reviewed the agenda, including the meeting objectives:

1. Promote and foster interdisciplinary consultation and collaboration among laboratory researchers, diagnosticians, and disease specialists and managers.
2. Provide an overview of recent and ongoing research on respiratory disease in domestic ruminants and wild sheep.
3. Identify knowledge gaps and future research needs and provide recommendations for research priorities to funding organizations.

Dr. Craig Stephen—Keynote Speaker

Rethinking Causal Relationships in Free-Ranging Wildlife

Dr. Craig Stephen, University of Calgary, is a faculty member at the Centre for Coastal Health where he studies the interactions of human, animal, and environmental health.

Causation has evolved throughout history and has included the following explanations: supernatural; imbalance; etiological; and epidemiological, including an imbalance between the patient and his or her environment. A cause and effect relationship can be difficult to determine since exposure does not guarantee illness, outcomes can be due to different causes, and outcomes can exist without a precise etiologic agent. Dr. Stephen used the example of cholera to illustrate his point. Ultimately, the cause of cholera was socioeconomics: the cost of water and miner labor laws.

Great gains have been made with vaccines and antimicrobials, which were the major focus of post-World War II research. However, more success has come from modifying the environment than from administering drugs. If health is promoted, the burden of disease can be reduced. Koch's postulates are useful for understanding mechanisms, but not for understanding the disease. Outbreaks of disease have more to do with populations than the agent that caused the disease. Not one variable can be identified as the cause of disease and the etiology is not always the weak point. Often, the weak point is the host biology, environment, or human constructs such as policy and culture.

When researchers worked to prove that smoking caused cancer, Bradford Hill developed his postulates, which accounted for multi-causal diseases. Dr. Stephen asked if wildlife could use this "smokers" gift. Epidemiologic methods were made for captive factors that can be counted and require that all cases are

found; proper controls are included; and that there is adequate follow up, time, and money. Not all of these requirements can be fulfilled in the wild.

Tools for fighting disease in the wild are limited to culling animals from the herd, modifying the environment, and educating the public. To implement a solution, researchers must provide enough evidence to support recommendations. Researchers are stuck in the legacy of Koch since bugs are easier to think about, communicate about, and fund. The result is, very little work has been done on the role of disease in the natural setting. Researchers need to transition from the study of disease to risk management—they need to think about perceptions, including values and sharing information; ecology, including risk and relationships; and qualitative methods that are inclusive of society.

The critical question for risk approach is, “What is the problem?” Several issues exist and they might be related, but they are not the same. Until a question is defined, the problem can’t be solved. Scientists often attack the etiology when there is a complex social problem.

Dr. Stephen asked participants, “When is harm realized?” This question is often tackled by a threshold approach, but when is the threshold reached and how is the threshold defined? Health is not the absence of disease, health is socially defined. Society decides when something is healthy and when it isn’t and different groups will always have different goals. Determining health needs a multi-method approach that includes many scientific disciplines and integrates information. A systems approach implies that solutions are not found in biology and pathophysiology alone; ecological change is only bad when society says so and solving scientific questions versus dealing with a wildlife management plan are not unrelated but are not the same.

Projects fail when culture and policy are not considered and when work is not completed from the ground up to build health and meet expectations. A cultural shift must occur. In order to solve problems those involved in wildlife management must provide research funds for evidence-based policy solutions, expand the concept of wildlife health specialists, and focus on decision making instead of on publishing. Lawyers end up solving environmental problems: science did not stop pollution, laws did. However, lawyers are informed by science.

Current tools are not able to provide the burden of proof for wildlife causation. New tools are needed and researchers need to start with the basics, including counting cases and determining a control. Researchers need to become problem focused, not disease focused and must become part of collaborative teams.

Dr. Vernon C. Bleich—Keynote Speaker

Ecology of Mountain Sheep:

Ramifications for Disease Transmission and Population Persistence

Dr. Vernon C. Bleich, CDFG (recently retired), discussed the ecology of mountain sheep and why researchers are concerned about disease transmission. Mountain sheep (big horn sheep) are highly specialized herbivores that are highly adapted to rough, steep, and open terrain. To evade predators, they use their acute vision to detect danger at long distances, and then seek refuge in places in which predators have difficulty capturing prey. Mountain sheep are gregarious creatures, which plays a role in their ability to detect and evade predators: large groups enhance the probability of survival by individual sheep and provide more alert individuals for detecting potential danger at any given time. The primary predator of mountain sheep is the mountain lion.

As do most polygynous ungulates, mountain sheep exhibit extreme sexual dimorphism, which likely evolved through sexual selection for large body size and larger horns among male sheep when compared to females. In order to enhance their probability of reproductive success, males tend to maximize body condition through the use of risky habitat. Females tend to take fewer risks to maximize the probability of

offspring survival. The sexes remain segregated for the majority of the year and the height of sexual segregation seems to be when offspring are most vulnerable to predation. As male lambs mature, they leave female groups and begin to associate with older males. During rut, virtually all social groups contain members of both sexes and males compete among themselves for access to mates.

The distribution of mountain sheep is reduced from historical levels. Dr. Bleich displayed a map depicting the approximate historical range of desert sheep in the United States outlined in yellow. The basic distribution of mountain sheep reflects a classic metapopulation structure. Dr. Bleich displayed a second graphic of the South–Central Mojave Desert Metapopulation. The graphic showed unoccupied ranges outlined in black, occupied ranges stippled in brown, and confirmed movements between subpopulations indicated by green arrows.

More recently, Epps et al. (in press) have predicted the probable routes of mountain sheep movements among subpopulations. Dr. Bleich displayed a graphic illustrating the relative probability of use of predicted travel corridors; the most likely corridors for gene flow among populations that have been reestablished by translocation; and impassable barriers, which have formed the basis for establishing the metapopulation boundaries used by CDFG in mountain sheep conservation planning efforts.

Using knowledge about the natural history of mountain sheep, researchers can draw some conclusions about how evolutionary history influences the vulnerability of mountain sheep to disease: mountain sheep are highly social and attracted to congeners; males travel long distances in search of reproductive opportunities; and mountain sheep exist in metapopulations, with males traveling between the subpopulations.

Dr. Bleich described a subpopulation of mountain sheep in the Sierra Nevada and the conservation efforts that have led to increased herd numbers. Maps illustrated the recovery units and herd locations. Currently, the greatest concern for conservation is the proximity of domestic sheep to mountain sheep. Following contact with domestic sheep and goats, mountain sheep may develop a number of contagious diseases, including respiratory disease and, as long as domestic sheep remain in proximity to mountain sheep in the Sierra Nevada, males have the potential to act as disease vectors.

Distance has played prominently into the assessment of contact risk relative to disease transmission from domestic sheep. Yet, movement distances rarely have been quantified within mountain sheep populations and buffer distances suggested by agencies are based on the straying distances of domestic sheep. To better understand the movements of mountain sheep, approximately 100 mountain sheep were captured and radio-collared over the past five years. Using GPS data and GIS maps, Dr. Bleich and his team developed a habitat suitability model, calculated habitat suitability scores, determined the home range size for 61 different animals, calculated the habitat suitability within home ranges, and determined the extent of the home range. Dr. Bleich displayed several graphics detailing the movements of two rams. Both examples confirmed that males travel great distances and are more likely than females to cross less suitable habitat. Such dispersal has tremendous implications for disease transmission, including the possibility for contact between mountain and domestic sheep.

Diseases of domestic livestock clearly are of concern to those charged with recovery of this endangered taxon. Although some argue that disease transmission from domestic to wild sheep has not been demonstrated, a conservative approach to this issue is dictated.

Shipping Fever and Calf Pneumonia in Cattle

Dr. Trevor Ames, DVM, University of Minnesota, reported on shipping fever and calf pneumonia in cattle. *Mannheimia (Pasteurella) haemolytica* is the dominant bacteria responsible for shipping fever, an historically ill defined entity that primarily occurs in young feedlot cattle. Dr. Ames displayed a photo of classic lesions of fibrinous necrotizing lobar pleuropneumonia.

Historically, shipping fever studies were flawed. Then, from 1985 to 1988, a large observation study involving 58,885 spring-born calves that entered a single feedlot was conducted. Complete necropsies were performed on all cattle that died and a diagnosis was assigned. From this study, researchers

determined that mortality due to fibrinous pneumonia ranged from 0.25% to 2.73% and proportionate mortality ranged from 10% to 57%. Peak fatal disease onset occurred within eight days of arrival to a feedlot, which drove the use of on-arrival, long-acting antibiotics. Further studies demonstrated that in years of high disease incidence the disease clustered within truckloads and within pens; the distance the calves were transported was never statistically significant.

Several facts are known about *M. haemolytica* and shipping disease: serotype 1 is the major serotype isolated from pneumonia lungs; the frequency of *M. haemolytica* isolations from nasal swabs is low at the farm of origin, greater at the auction barn, and markedly higher at the feedlot; serotype 2 is the predominant isolate at the farm while serotype 1 is predominant at the feedlot; rapid transmission of *M. haemolytica* between calves at the time of commingling and within feedlot pens has been demonstrated; and a critical number of *M. haemolytica* must be inhaled even with prior viral infection before pneumonia will occur.

Many viruses have been implicated in shipping fever and, when present, make the disease more severe and harder to treat. Experimental challenge models for *M. haemolytica* can be exacerbated by the addition of a variety of different respiratory viruses. In addition, seroconversion to a number of viruses is common in beef calves with shipping fever; low antibody titers on arrival at the feedlot, for some viruses, are a risk factor for respiratory disease; and the most common virus isolated from cattle dying of bovine respiratory disease is bovine viral diarrhea virus (BVDV).

Only *M. haemolytica* serotype 1 replicates to large numbers in the upper respiratory tract of calves and results in naturally occurring disease. Therefore, serotype 1 must have a phenotypic advantage. Using the antibiotic tilomicosin decreases the number of calves that culture positive for *M. haemolytica* in the upper respiratory tract and the number of organisms that can be cultured from positive calves.

Dr. Ames displayed photos of alveolar macrophages, which stimulate the recruitment of other macrophages and numerous neutrophils. Marked neutrophil exudation into the alveolar space precedes the extensive tissue damage and neutrophil depletion in calves prior to experimental challenge with *M. haemolytica* prevents the development of the characteristic pneumonic lesions. In addition, hallmark lesions are mediated through the disruption of endothelial cells, which leads to exudation, thrombosis, and necrosis.

Evidence indicates that *M. haemolytica* ST1 leukotoxin plays a role in lesion formation. Photos of lung tissue from calves challenged with the mutant strain of *M. haemolytica* (which is unable to produce leukotoxin) depicted very little lesion formation in the lungs. All cattle challenged with the mutant strain survived, compared to calves receiving the wild strain of *M. haemolytica* (which produces normal amounts of leukotoxin), further indicating that leukotoxin is a major contributor in the pathogenesis of lung injury in shipping fever pneumonia.

Treatment for shipping fever is based on the presence of fever plus or minus other symptoms. A definitive diagnosis is made by culturing, pathologic testing, or immunohistochemical staining.

Pasteurella multocida plays a smaller role in the development of shipping fever, but is an important cause of enzootic pneumonia of cattle younger than 6 months of age. *P. multocida* A:3 is the most common isolate from feedlot cattle dying of pneumonia. Dr. Ames challenged calves intratracheally with *M. haemolytica* and *P. multocida*. Results were dose dependent where log phase cultures were much more pathogenic for *M. haemolytica*. Interestingly, log phase culture of high doses of *P. multocida* produced minimal lesions, suggesting it is unlikely a primary pathogen. More importantly however, was the finding that the *P. multocida* consistently overgrew lesions produced by *M. haemolytica* even in calves not exposed to *P. multocida*.

Further evidence indicates that *P. multocida* plays a role in the formation of lesions and severity of illness. Several facts are known about *P. multocida* including that it colonizes in the upper respiratory tract in the presence of chronic impairment of the respiratory tract defenses and its capsule, which aids in resistance to phagocytosis, and its lipopolysaccharide (LPS), which stimulates neutrophil influx and the inflammatory response, may produce significant lung lesions. This organism may overgrow existing pulmonary lesions established by other pathogens. Lesion size is affected by on-going damage to the respiratory defenses that allows organisms to continue to multiply.

Development of enzootic calf pneumonia is caused by three factors: etiologic agents, including the presence of possible viral and bacterial agents; calf factors, including their immune status, age, status of their respiratory defenses, and level of nutrition; and environmental factors, such as temperature, humidity, air flow, amount of fresh air, and the amount of direct contact between calves. Environmental factors are especially important and calves raised in inadequate housing, which occurs in the majority of calf barns, have significantly poorer growth rates and more problems with enzootic calf pneumonia than calves raised in housing considered adequate.

Disease onset is within two weeks of birth, but is hard to identify. Pathologic features include a chronic, progressive lesion; purulent or exudative bronchopneumonia; cranioventral distribution; abscessation, necrosis, and bronchiectasis (may be present); and consolidation (hepatization). Pathogenic events begin with impaired respiratory defenses and inhalation of the organism. The organism proliferates in the lung though resistance to phagocytosis and lesions develop from LPS.

Researchers at the University of Minnesota have evaluated various experimental and commercial vaccines. In summary, higher antibody levels against leukotoxin alone consistently correlate with reduction in pneumonic lesion scores. Practical methods of reducing shipping fever include vaccinations for *M. haemolytica* and *P. multocida* that are used in combination with long-acting antibiotics given on arrival; viral vaccines; and the purchase of healthy calves that are treated for parasites, placed on a good nutrition program, and housed in well-managed pens. Practical methods for reducing enzootic calf pneumonia include proper housing and management.

Future topics for *M. haemolytica* research include leukotoxin–cell interaction, inflammatory cascade regulation, and protective immunogens. Future directions for *P. multocida* research include the identification of critical control points for calf pneumonia and the identification of immunogens which induce protective immunity for *P. multocida*.

Respiratory Disease in Domestic Sheep

Dr. Mike Sharp, Veterinary Laboratories Agency (Edinburgh, Scotland), presented an overview of respiratory disease in domestic sheep. Several agents, in a variety of combinations, are responsible for respiratory disease in domestic sheep and different combinations can cause acute, subacute, or chronic infections.

Atypical pneumonia is the most common pneumonia in young sheep, but clinical signs are often mild and the disease is not detected until the animal is slaughtered and the lungs are examined. However, symptoms are much worse when *Mycoplasma ovipneumoniae* is combined with *Mannheimia haemolytica*.

Persistent viral infections in domestic sheep include jaagsiekte sheep retrovirus, a contagious lung tumor, and maedi visna virus, a progressive interstitial pneumonia. Antibodies to acute viral infections are common, indicating that infection normally occurs within a few weeks of birth. However, the range of systemic signs is very short and typically respiratory signs may continue when the virus no longer can be isolated. New molecular tools may improve viral detection.

Pasteurellosis and *M. haemolytica* have dominated respiratory disease in sheep. Originally identified in 1932, *Pasturella haemolytica* has been further classified as *P. trehalosi* and *M. haemolytica*. Found in nearly all healthy sheep, *M. haemolytica* can be isolated mainly from the nasopharynx and *P. trehalosi* mainly from the tonsils.

Pasteurellosis caused by *M. haemolytica* has several different presentations that include septicemia of young lambs, pleurisy and pericarditis of older lambs, and acute or subacute pneumonia in older sheep. Predisposing factors for pasteurellosis may include environmental stressors, but there is no structured epidemiological data to support this hypothesis. Much better data exists in terms of other infectious agents that cause the illness. Pneumonic pasteurellosis is a sporadic disease and recurring problems suggest an underlying factor such as a lung tumor or the presence of PI3. The role of PI3 has been

proven through a reliable experimental model of infecting lambs with PI3 and then exposing them to *M. haemolytica*; the disease was produced in 90% of these lambs.

A wide range of antibiotics are effective against *M. haemolytica* and a vaccination, which offers the best means of protection, is now commercially available throughout the United Kingdom and Europe.

The phenotype of an *in vitro* *M. haemolytica* organism is very different from that of an *in vivo* organism, indicating that the phenotype is influenced by the host. Therefore, the repertoire of *M. haemolytica* genes that are expressed *in vivo* in sheep must be identified. The *M. haemolytica* genomic sequence is now available and will aid identification of *in vivo* gene expression, novel molecules and pathways, and gene regulation. Future research must include a global approach, combining genomics and proteomics, to analyze the structure, function, and interactions of the proteins produced by *M. haemolytica* in sheep.

Molecular Basis for the Enhanced Susceptibility of Bighorn Sheep to Pneumonia: How much do we know?

Dr. Sri Srikumaran, Washington State University, opened his presentation by stating that it takes more than one university to crack the problem of respiratory disease in sheep.

Mannheimia is a common commensal of the nasopharynx in bighorn sheep, domestic sheep, goats, and cattle. Most neonates acquire the organism from their parents within one day of birth. Although there is evidence that domestic sheep transmit the disease to bighorn sheep, bighorn sheep can develop pneumonia and die without contact with domestic sheep. Researchers must understand why bighorn sheep are so susceptible to pneumonia before they can develop meaningful control measures.

In the respiratory tract, cilia on the mucosal surface of the trachea are the first line of defense against bacterial pathogens, and are responsible for moving particles out of the tract. Predisposing factors such as stress or a viral infection may cause the organism to shift from being a commensal to a pathogen. When a viral infection exists, the mucosal lining is degraded and the infection can move to the lower respiratory tract. Remaining defenses fail to resist infection because of virulence factors of the bacteria, including leukotoxins (Lkt). The polymorphonuclear leukocytes (PMN) of bighorn sheep are much more susceptible to Lkt than PMNs of domestic sheep, which may explain why bighorn sheep are more susceptible to *Mannheimia/Pasteurella* pneumonia.

Beta-2 integrins are the host cell receptors for Lkt on the white blood cells of bighorn sheep, domestic sheep, and cattle. Integrins are adhesion molecules that facilitate intercellular communications. Beta-2 integrins are responsible for regulating leukocyte traffic, interactions between lymphocytes, phagocytosis, and cytolysis.

Research indicates that bighorn sheep susceptibility is not due to a difference in their PMN receptor sequence or receptor expression. Other possible factors include a difference in toxin binding, a difference of innate membrane susceptibility, and a difference in signaling events.

Bighorn sheep infected with a mutant *M. haemolytica* A1 that does not produce leukotoxin did not die, but did develop mild lesions in the lungs, which suggested that other milder factors might play a role. One possible factor is lipopolysaccharide (LPS). LPS can cause disease by itself and is known to complex with Lkt. The receptor for LPS is CD14. Future research will include determining if LPS and CD14 play a role in enhanced susceptibility of bighorn sheep PMNs.

Dr. Srikumaran and his team determined that a strain of *M. haemolytica* A1 that does not cause pneumonia in domestic sheep does cause pneumonia in bighorn sheep and may be an organism that is transmitted from domestic sheep to bighorn sheep.

Future research should include studies of lung defense mechanisms of bighorn sheep and domestic sheep, which may include differences in cytokine production, phagocytosis, or intracellular killing. Research should also include examining any viruses or other pathogens that might be involved in disease transmission from domestic sheep to bighorn sheep.

Session I: The following are the key discussion points from Session I Q/A. Panel members included Dr. Stephen, Dr. Bleich, Dr. Ames, Dr. Sharp, and Dr. Srikumaran.

- The effective dose of infecting agent used in lab trials is always around 1×10^9 , so the bighorn sheep in the wild would need to get a dose equivalent to that for disease transmission to occur. But, if researchers consider the evidence that Dr. Srikumaran presented, the necessary dose may be much smaller. In addition, there might be sheep to sheep transmission within the group that would be similar to transmission in a feedlot. Research has shown that the susceptibility of the host changes the effective dose.
- Members of the audience noted that they are not seeing the variety of pneumonia they used to see and would be interested in knowing what types of pneumonia others are seeing.
- Long-acting antibiotics are actually more effective if cattle are treated after they arrive at their destination; however, the improved results could be a management issue. If calves are going to be in transport for a long period of time, then it is better to treat them before they get on the truck.
- Both *M. haemolytica* A1 and A2 strains can kill domestic sheep and the A1 strain used by Dr. Srikumaran was isolated from cattle. The control used was the parent from which the mutant was derived.
- *M. haemolytica* A1 can be transmitted from cattle to sheep and can kill sheep. However, die offs in bighorn sheep tend to be associated with domestic sheep because the affinity between bighorn sheep and cattle is small.
- Dr. Srikumaran will be conducting tests next year to determine the distance necessary for disease transmission between domestic sheep and bighorn sheep. Some participants believed that nose to nose contact was necessary for transmission, but others said that transmission could occur if grazing territory overlapped. Studies in children have discovered viruses can be transmitted from one child to another through toys.
- Goats have been implicated in disease transmission to bighorn sheep. No such evidence has been linked to llamas, elk and deer.
- Wild type organisms are far easier to transmit than the mutant that was unable to produce leukotoxin. Data indicate that leukotoxin genes are constitutively expressed, but the tonsils and nasopharynx may not harbor enough organisms to cause disease. Some virulent organisms benefit from an increased virulence since a less mobile host that is shedding bacteria may be more effective at transmitting the organism.
- Discussions concerning the ability to isolate *M. haemolytica* from dead bighorn sheep ended in discrepancy. Some members of the panel and audience felt that *M. haemolytica* was difficult to culture, while others had not had any problems. If the correct culturing techniques aren't used, the culture may not grow, but that doesn't mean the organism isn't present.
- Dominant organisms change over time, but could actually be a group of organisms acting together. Dr. Sharp added that he would like to get a feel for the proportion of wild sheep that are already carrying these agents. If domestic sheep are a reliable model, then bighorn sheep may also have a commensal relationship with the organism that eventually causes disease. Dr. Sharp is interested in the mechanisms involved that "tip the scale." Dr. Srikumaran replied that current research being conducted could answer Dr. Sharp's question.
- When there is an outbreak of respiratory disease in bighorn sheep, there are two patterns: widespread die-offs vs. death of some members of the herd followed by sporadic deaths of lambs for several subsequent years.
- A member of the audience noted that the most frequently offered solution is to reduce the contact between domestic sheep and bighorn sheep, but suggested that if that isn't a critical control point, perhaps there are other solutions to this problem. Regardless of the agent responsible, is reducing contact the only method of reducing the problem? The panel acknowledged that disease can occur without contact between domestic and wild sheep.

Pasteurellaceae and Other Microorganisms Involved in Respiratory Disease

Dr. Glen Weiser, Caine Veterinary Center - University of Idaho, discussed the microorganisms involved in respiratory disease. In 1938, *Pasteurella* spp. and *Corynebacterium pyogenes* were implicated in respiratory disease, both of which are still consistently isolated from the lungs of bighorn sheep that have died from pneumonia. Recent research has focused on *Pasteurella*, some of which have been renamed *Mannheimia* spp. However, researchers have realized that *Pasteurella* alone does not answer the questions.

Dr. Weiser displayed a photo of agar plates and culture tubes. A photo of isolated colonies on an agar plate illustrated beta hemolysis. The *P. trehalosi* shown did not exhibit beta hemolysis. Several bacteria were associated with wild sheep in the western United States and Canada from 1986–2005. *P. trehalosi* was cultured from 84% of healthy and 16% of diseased animals, *Mannheimia* spp. were cultured from 85% of healthy and 15% of diseased animals, and *P. multocida* was cultured from 73% of healthy and 27% of diseased animals. Several major biovariant types were associated with the *Mannheimia* spp. When organisms are isolated from the lungs of a host that died from pneumonia, a mix of organisms is usually found.

Dr. Weiser tested superoxide dismutase as a virulence factor in Pasteurellaceae. Of 107 *P. multocida* tested, 94 were *sodC* positive and there was only one unique allele; of 25 *P. trehalosi* tested, 21 were *sodC* positive and there was only one unique allele; of 117 *P. haemolytica* tested, 62 were *sodC* positive and there were eight unique alleles; and of 13 *Histophilus somni* tested, 1 was *sodC* positive and there was one unique allele. Dr. Weiser is still working on the killing assays to determine if there are any virulence differences.

When testing leukotoxin as a virulence factor in *M. haemolytica*, five *lktA* alleles were identified using restriction fragment length polymorphism (RFLP).

Several bacteria are worthy of additional study and include the following: *Streptococcus*, *Staphylococcus*, *Arcanobacterium pyogenes*, *Moraxella*, *Neisseria*, and *Mycoplasma*. Several rare isolates, found mainly with non-culture methods, may also play a role: *Actinobacillus*, *Histophilus*, *Bordetella*, *Pseudomonas*, and *Clostridium* or *Fusobacterium*. Several species of *Streptococcus* and *Staphylococcus* were isolated from wild sheep in Idaho in 1994. Isolated *Streptococcus* species included the following: *acidominimus*, *bovis*, *mitis*, *mutans*, *suis I*, *sanguis*, and Group E. Isolated *Staphylococcus* species included the following: *auricularis*, *cohnii*, *epidermidis*, *sciuri*, *warneri*, and *xylosus II*.

In 2006, additional species were identified by non-culture methods: *Streptococcus pneumonia*, *Staphylococcus aureus*, *Clostridium* spp., *Sphingomonas wittichii* (soil bacteria used in Japan as a bioremediation bacteria that is able to use toxic pollutants as a carbon source), and *Fusobacterium necrophorum*. Non-culture methods suggest a greater diversity in several general than are typically found by culturing.

The following organisms were identified by culture in the 1995–1996 Hells Canyon die-off: *P. multocida*, *P. trehalosi*, *M. haemolytica*, *P. gallicida*, *M. ovipneumoniae*, *A. pyogenes*, *Enterococcus* spp., *Streptococcus* spp., *Acinetobacter* spp., *Enterobacter* spp., *E. coli*, *Moraxella* spp., *Proteus* spp., and *Pseudomonas* spp. Several viruses were also identified.

Dendritic cells recognize microorganism-associated molecular patterns (mamps), but when a commensal mamps is recognized, it does not create an inflammatory response (that creates an infection). According to Dr. Weiser, future research efforts should ask if commensal and pathogen mamps could be characterized and used to identify the most pathogenic forms and do some commensal mamps possess properties that allow them to become pathogens. Furthermore, because mamps are recognized by pattern recognition receptors, could the study of these receptors in wild sheep help researchers understand the potential for disease from commensals?

Viruses as Predisposing Factors to Bacterial Pneumonia

Dr. Howard Lehmkuhl, National Animal Disease Center, acknowledged that respiratory disease is a “complex” complex where death is sometimes the first sign of illness. Respiratory disease is multifactorial in that it involves stress, viruses, and bacteria, and polymicrobial in that it typically involves a combination of viruses and/or bacteria. Innate pulmonary immunity includes mucociliary clearance, immunoglobulins, pulmonary surfactants, alveolar macrophages, and antimicrobial peptides. Stress factors for the host include environmental temperature fluctuation, dust, dampness, injury, hunger, dehydration, nutritional deficiencies, fatigue, social organization, castration, and tail docking.

There are a variety of chronic and acute viruses associated with sheep respiratory disease in the United States. Ovine progressive pneumonia virus and pulmonary adenocarcinoma virus, which is very rare cause chronic disease whereas parainfluenza-3 virus, respiratory syncytial virus, bovine viral diarrhea virus, adenoviruses (eight serotypes known in sheep and two in goats), and bovine herpes virus-1, which only occurs in sheep following neonatal vaccination are associated with acute disease. Parainfluenza-3 virus and respiratory syncytial virus have been isolated from bighorn sheep. Antibodies to bovine viral diarrhea virus and the ovine and caprine adenoviruses have been demonstrated in bighorn sheep. Some viruses, such as the ovine, and caprine, adenoviruses, are part of a small niche market, so a vaccine will probably never be developed for them.

There are also several bacterial agents associated with sheep respiratory disease. The primary agents that have been mentioned today include *Mannheimia haemolytica*, *Pasteurella multocida*, and *P. trehalosi*. *Bordetella parapertussis* and *Arcanobacterium pyogenes* can predispose domestic sheep to *P. multocida*.

Viral infections alter host defense mechanisms by direct immunosuppression, direct tissue damage, disruption of the mucociliary clearance system, alteration of macrophage and neutrophil functions, production of pro-inflammatory cytokines that cause a cascading event leading to further tissue damage, alteration of surface epithelial cells to favor bacterial adherence and growth, and the release of iron enhancing bacterial growth and colonization. Direct tissue damage is the primary factor and seems to self-perpetuate once it occurs.

Virus exposure can occur without infection, especially in young animals with higher levels of maternal antibodies. When virus infection occurs, the typical result is a subclinical infection. Where clinical disease results, it can be mild, moderate, or severe that occasionally ends in death. Common clinical signs include nasal exudates, ocular discharge, cough, decreased appetite, fever, respiratory distress, and listlessness. Clinical signs and lesions are more severe with a secondary bacterial infection. It is difficult to identify underlying viral infections once the bacterial infection is established.

Viral virulence factors depend greatly on the viral strain; infecting viral dose; route of infection (aerosol or direct contact); and age, immune status, and condition of the host. Lesions can be macroscopic, such as atelectasis/consolidation or ulceration, or microscopic, such as nuclear and cytoplasmic inclusions.

Dr. Lehmkuhl displayed several photos:

1. Lung from a lamb inoculated with ovine adenovirus strain RTS-151 with consolidation in the lower half of some or all lobes.
2. Hyperplasia and cytomegaly of bronchiolar epithelium of a lamb inoculated with ovine adenovirus strain RTS-151. Lumen contains desquamated epithelial cells and neutrophils. Some cells contain intranuclear inclusions.
3. Bronchiolitis-alveolitis in a lamb inoculated with ovine adenovirus type 5. Neutrophils, necrotic epithelial cells, macrophages, and fibrin filled some bronchioles and alveoli. No obvious intranuclear inclusions were observed.
4. Lung from a lamb infected with respiratory syncytial virus with multiple foci of interstitial pneumonia involving all lobes of the lung.

5. Bronchiolitis and parabronchiolitis in a lamb inoculated with respiratory syncytial virus. Sloughed and disrupted bronchiolar epithelium are evident as well as accumulation of mononuclear cells (macrophages).
6. Lung from a lamb inoculated with parainfluenza-3 virus with multiple irregular areas of consolidation.
7. Terminal bronchiole from a lamb inoculated with parainfluenza-3 virus with multiple viral inclusions in the cytoplasm of epithelial cells and epithelial cell hyperplasia.

Diagnosis can be made with electron microscopy, virus isolation, serology, antigen detection, and PCR. The most important step is virus isolation, allowing for further analysis of the infection virus.

Future research needs include acquiring timely samples, developing bighorn sheep cell cultures, determining the prevalence and pathogenicity of viruses isolated from bighorn sheep, and fulfilling Koch's postulates. We know parainfluenza-3 virus and respiratory syncytial virus have been isolated from bighorn sheep pneumonias and that bovine viral diarrhea virus antibody and sheep and goat adenovirus antibody to all but ovine adenovirus-1, -6, and goat adenovirus-2, have been found in bighorn sheep. The role these viruses play in pneumonia is not currently understood.

Pathologic Examination of Bighorn Sheep Pneumonia—Advantages and Limitations

Dr. Kathy Potter, Washington Animal Disease Diagnostic Laboratory (WADDL), was asked to study lamb mortality in bighorn sheep in the Hells Canyon. Study protocol included necropsy and histopathology, bacteriology, virology, and immunohistochemistry. Pathologists at WADDL have examined many bighorn sheep, nearly all of which were from Hells Canyon. However, most were dead and were not in the best condition. For this study, interest had shifted to studying lamb mortality and the relationship between lamb mortality and recent massive die-offs in adults.

A plan was developed that included the collection of lambs during early stages of the disease and administration of the following tests: immunohistochemistry for several viruses, adenovirus PCR, virus microarray for discovery, virus isolation on bighorn sheep cells, and 16S bacterial population survey. The goal was to collect lambs as early as possible during a pneumonia outbreak and to harvest specimens that were in the best condition.

Three populations were identified as having lamb mortalities and researchers were able to collect 11 lambs. Seven of 11 lambs were killed and necropsied; 6 were collected and necropsied in good post mortem condition. Many already had advanced bronchial pneumonia.

Dr. Potter displayed a photo of a typical dead lamb in the wild—it was already severely dehydrated. Dr. Potter also displayed a photo of a section of lung from a poorly preserved lamb; purple areas indicated airways filled with bacteria. The next slide (from a freshly killed lamb) showed tissue with clear signs of bronchial pneumonia. Once necropsied, most lambs were discovered to have extensive pneumonia.

Dr. Potter then displayed a variety of photos of tissue samples and lungs that illustrated the different degrees of infection of the lambs that were harvested. In the freshly harvested animals, a junction between the infected and unaffected lung could be seen. Later slides showed alveoli that were filled with neutrophils. A small amount of hyperplasia could be seen, which could be caused by the involvement of a *Mycoplasma* in these infections. Several more photos illustrated the affects of infection on the bronchioles. Later in the season, lambs that were ill longer before they were harvested started to develop abscesses, lots of consolidation, and necrosis.

Interestingly, researchers could identify sick lambs because of the way the lambs held their ears. Further research indicated that most of the sick lambs had otitis media and the middle ears were filled with purulent exudate. Dr. Potter displayed three photos detailing the otitis media.

Several ancillary tests were conducted on the freshly harvested lambs. Results indicated that the dominant culturable bacteria found in the lambs varied by location. Serology results indicated low titers to bovine respiratory syncytial virus and PI3. There were discordant results for PI3: PCR in all lambs was

negative, but by immunohistochemistry all were positive for PI3. Results for remaining tests are still pending.

Dr. Potter displayed a slide from the positive control lamb that was infected with PI3. The bright red staining is indicative of PI3.

From this project, Dr. Potter learned that it is useful to collect specimens that are as fresh as possible and early in outbreaks, researchers should snap-freeze fresh tissues for nonculture methods, and the possibility of doing inoculation studies should be considered if there is a putative study agent.

Does a Primary Infectious Agent Underlie Bighorn Sheep Pasteurellosis?

Dr. Tom Besser, WADDL, discussed the possibility of primary infectious agents that may predispose bighorn sheep to pneumonia. Dr. Besser displayed a figure that illustrated the absence of any specific *Pasteurella* type that is common to pneumonia in bighorn sheep; instead the diverse strain types isolated from pneumonic sheep were generally similar to the diverse *Pasteurella* types in the nose of apparently healthy bighorn sheep. Is there a predominant agent that causes the initial illness and makes the host more susceptible to inhaling an agent that causes pneumonia or is there an agent that is causing death that has not yet been isolated and identified?

Dr. Besser conducted a 16S bacterial population survey in bighorn sheep pneumonia specimens using a protocol developed by Dr. Pace from the University of Colorado. Results on single lambs from Sheep Mountain, Imnaha, and Black Butte identified *Mycoplasma ovipneumoniae* as composing about 32%, >75%, and 21% respectively, of the bacteria in the lung washings. In four additional lambs from these herds with more advanced pneumonia, obligate anaerobic bacteria dominated the lung washing flora.

PCR was then used to look specifically for *M. ovipneumoniae*, and it was found to be present in the lung tissues of all seven lambs used for the 16S study. All PCR products were confirmed by sequencing. Two sequence variants were detected in equal numbers, indicating the *M. ovipneumoniae* likely has two divergent 16S operons in its chromosome (as is the case with several other mycoplasma species).

M. ovipneumoniae exhibits different colony morphology than most mycoplasma and was not recognized by laboratory staff on initial cultures. Repeat cultures successfully identified *M. ovipneumoniae* and one isolate grew quite well.

Dr. Besser conducted a retrospective case-control study. Test subjects were all adult bighorn sheep, half with and half without bronchopneumonia. DNA was extracted from paraffin embedded lung tissues and tested for *M. ovipneumoniae* by PCR; presence was confirmed by sequence analysis. Results indicated that 17 of the 22 samples were positive for *M. ovipneumoniae*. There was no detectable difference according to bronchopneumonia status and all tested animals came from populations with a history of lamb and/or adult pneumonia.

The exposure of bighorn sheep to *M. ovipneumoniae* was tested by detection of specific antibodies in serum samples. When populations without a history of pneumonia were compared to populations with a history of pneumonia using passive hemagglutination serology, those populations without pneumonia were consistently seronegative for *M. ovipneumoniae*. Serum samples from the Lostine herd in Oregon were seronegative prior to a pneumonia outbreak in 1986; subsequent samples from the same population were seropositive after the outbreak. A graph comparing *M. ovipneumoniae* antibody GMT in herds with and without pneumonia outbreaks clearly illustrated the correlation between the presence of antibody and a history of pneumonia in the herd.

In conclusion, *M. ovipneumoniae* is strongly associated with the occurrence of bronchopneumonia in bighorn sheep at the population level. In adults, *M. ovipneumoniae* is more consistently identified in the lungs of both lambs and adult bighorn sheep with pneumonia than any single *Pasteurella* spp. strain. In lambs, *M. ovipneumoniae* alone may be sufficient to cause fatal bronchopneumonia. In adults, secondary bacterial agents are apparently always present and probably contribute significantly to disease. The agent is presumably introduced into bighorn sheep populations from an external source, but long-term carrier

bighorn sheep cannot be ruled out at present. The agent appears persistent in populations once it is introduced, but transmission may be limited in some cases.

Future plans include a comparative serology, a *M. ovipneumoniae* challenge of neonatal bighorn sheep lambs, experimental transmission between bighorn sheep and from domestic sheep to bighorn sheep, and intervention possibilities via antibiotics or vaccination.

Session II: The following are the key discussion points from Session II Q/A. Panel members included Dr. Weiser, Dr. Lehmkuhl, Dr. Potter, and Dr. Besser.

- Researchers on the panel had not looked at domestic sheep, so they could not conclude if *Mycoplasma* was coming from the domestic sheep, but determining if the infectious agent was coming from domestic sheep could be tested. Dr. Sharp noted that *M. ovipneumonia* was common in sheep.
- A member of the audience noted that during severe *Mycoplasma* outbreaks that occasionally occurred in closed herds, many of the animals also had otitis media (*Mycoplasma* were present in the ears) and ear mite infections; treating for ear mites was somewhat effective.
- There is evidence in the literature that mycoplasma can be a threat to domestic sheep, especially when multiple strains are present; therefore, in combination with the higher susceptibility of bighorn sheep to leukotoxins, it could be possible that even a single strain of mycoplasma could be a pathogen for bighorn sheep.
- PI3 is an important agent for pneumonia in lambs and a positive PI3 histochemistry is seen in pneumonic and nonpneumonic adult bighorn sheep, although it could be something that is cross reacting to look like PI3 as well.
- The lambs studied in Hells Canyon were between 10 days and 6 weeks old. Mortality peaked at 6 weeks of age, but researchers were surprised to see lambs as young as 10 days that already had pneumonia even though they looked fine (no drooped ears, etc.) As for body condition, in general, their score was good.
- Mycoplasma has been found in herds exposed to domestic sheep.
- Lamb pneumonia tends to occur during a short time period in the summer and has a very high mortality (typically by 8 weeks). In adults, pneumonia is more sporadic, but tends to occur in the fall and early winter in Hells Canyon.
- Non-culture, nonspecies methods are appealing and should be used in conjunction with other methods to get a broad picture; then researchers can very carefully select which systems to use. Selection must be based on current science and some intuition.
- Antibodies seem to be a better indicator of exposure, not of immunity. As far as viruses are concerned, type-specific immunity is seen. Durable immunity is hard to get when dealing with many serotypes, which is why it is hard to create a vaccine. PI3 might offer durable immunity for a year or two, but the immunity is not indefinite.
- The lambs that were studied in Hells Canyon were becoming sick before colostrum antibodies would be dying off. Additional research has indicated that the lambs were not getting antibodies from the mother. A member of the audience suggested the lambs could have been born with the disease.
- A member of the audience asked if researchers were getting confused by chasing the bugs considering there are so many and would researchers change to managing the disease risk instead. Vaccines and antibiotics are an alternative to management. Another member of the audience agreed that in the dairy industry, managing housing is much more effective and the better approach. The panel responded that if the interest is in knowing whether or not exposure to domestic sheep is the issue, then the organism causing the illness needs to be identified.
- Field practitioners need standardized protocols for collecting samples. Dr. Gonzales added that field practitioners need to know what is happening and what the mechanisms are when they get to a disease outbreak.

- Dr. Potter noted that when she first saw the tissue slides, she thought the illness was caused by *Mycoplasma*.
- These conversations occur over and over again and vaccines and antibiotics are never going to cure the problem. What is the ultimate study—finding the bug or defining management between domestic and bighorn sheep? Another member of the audience asked about the role of translocation.

Dr. Gonzales provided a brief wrap-up of Day 1 presentations and discussions, and adjourned the workshop until 8am on Day 2.

Workshop Day 2

Welcome

Dr. Jessup welcomed participants to the second day and shared a story about sea otters and how several agencies have worked together to share resources and expertise. Each agency and organization divvied up the work and collaborated together to study all aspects of the problem. In 12 years, tremendous progress has been made and resources have grown because their success has attracted more funding. The lesson: mutually supportive groups make a lot of progress.

Susan reviewed the working group process, composition and meeting locations throughout the building. Participants were adjourned to work the rest of the morning in their working groups.

Working Group Reports

After lunch, participants reconvened in a plenary session to hear reports from each working group. Working group leaders/co-leaders presented the results: Dr. Gonzales (Working Group C), Dr. Garde (Working Group A), Dr. Cassirer (Working Group B) and Dr. Jessup (Working Group D).

Working Group C

Working Group C was tasked with three objectives: 1) Attempt to develop a disease outbreak diagnostic protocol that would provide conclusive evidence of disease transmission from newly introduced bighorn sheep or domestic sheep to resident mountain sheep in an outbreak area (is it possible); 2) Identify and discuss the merits of applying current/potential diagnostic practices and tools prior to and during an outbreak to determine root causes of respiratory disease; and 3) Identify research needs to develop and implement potential diagnostic tools. Working Group C hoped to complete the following outcomes: a proposed disease outbreak diagnostic protocol adequate to provide conclusive evidence of disease transmission; a list of current and potential diagnostic practices and tools that could be used prior to and during an outbreak event to aid in determining the root causes of respiratory disease; and a list of potential diagnostic tools, and the research needed to develop and implement them.

Summary

Throughout the workshop, participants have debated the necessity of “identifying the bug” responsible for respiratory disease in bighorn sheep. From a management point of view, the manager needs to be able to identify the “bug” and the healthy populations; what else is there to look at? In addition, decisions need to be based on the best available science, including the agent responsible for disease.

Sampling problems include collection, transport, and storage of samples. Researchers have not been able to adequately study viruses and won't be able to until they know how to collect viral samples.

Outbreak Protocol

Working Group C completed an excel spreadsheet concerning a standard outbreak protocol (Appendix E). Not every item on the list can be fulfilled; some are very resource dependent and others cannot be performed at this time. The working group used this opportunity to identify needs and will figure out who and how at a later date. Some of the virology protocols were eliminated based on expert opinion. Currently, viral samples taken in California are sent to some unknown lab and are often lost. Researchers first need to locate a reliable lab for viral testing.

Several important items were included in the spreadsheet. Limited information can be obtained from dead carcasses that are not fresh. Live animals must be collected to get the best information on disease outbreak; even when live animals are harvested, their illnesses are often well advanced.

Research Needs

Research needs included the following: virus isolation at primary low-passage lines; collection, transport, storage methods; a centralized sample bank and information; non-culture-based sampling methods; and sample plan development. Sampling protocols need to be simplified or pathologists will not perform the tests.

It was noted that Caine Veterinary Center has a tremendous archive of *Pasteurellaceae* that may be at risk. The working group suggested finding a place to house and maintain the collection.

Question/Answer

After the presentation by Dr. Gonzales, the following issues were discussed:

- There was discussion about the potential for training hunters to collect samples of dead sheep they encounter in the wild in an effort to increase sample sizes. Members of the audience noted both success and failure with having members of the public collect samples. Success varies a lot with each hunter and how interested he or she is in the project. The first step would be to educate hunters about what is needed and the second step would be to make collection easy for them. Submitting a collection should be a requisite to obtaining a tag. It was reported that Montana has had a lot of success, including when they asked hunters to return the entire gastrointestinal tract. Some participants shared their experience that outfitter/guides were not as interested in sample collection, since they often hunted in some very rugged terrain and remote areas.
- It was also suggested that veterinary students be enlisted to help, especially if states could set up a check station.
- A field protocol is very important and should address biologists in remote areas, and include a requirement for photographs of the carcass.
- To date, not everyone may buy into the same protocol, but this is a good starting point for consistency across the board. The following discussions are still needed: what tests should be done in the same laboratory and does the entire community need to agree on one lab.
- There should be no difference in sample collections between field and lab necropsy.

- Not all domestic sheep operators allow researchers to take samples from domestic sheep that have died of natural causes, which is where the cooperation from domestic sheep groups becomes necessary.

Working Group A

Working Group A was tasked with two objectives: 1) Identify the critical unknowns/obstacles that prevent consensus on the contribution of diseases acquired from domestic sheep on wild sheep and 2) Discuss methods of overcoming these obstacles through robust study design in the natural setting. Outcomes for Working Group A included the following: a graphic representation of the network of factors that influence disease transmission and the subsequent impacts, within and between wild and domestic sheep; a prioritized list of the critical uncertainties in the identification of transmission events between wild and domestic sheep; and a list of practical field methods to address the critical uncertainties when designing the “ultimate study” on disease transmission between species.

Ultimate Study

Working Group A developed a causal/network diagram (Appendix C). Risk occupied the center of the diagram since members of the working group kept coming back to risk and realized there were several obstacles to risk, including the biology of domestic sheep and variables associated with non-domestic sheep. Limitations in field trial design included finding cases, classifying herds, assigning controls, and applying ethics and access to animal use. Finally, one study is not necessarily going to provide the needed proof.

Members of the working group realized that they couldn't address the “ultimate study” until they addressed sociological problems. Two areas of sociology need to be considered: 1) the determinants of perception, including problems in data perception, issues surrounding communication, and belief systems and 2) the consensus of goals the burden of proof (see Figure 2 in Appendix C).

Part I: A Rigorous Sociological Study of Cross-Cultural Consensus

- Who—politicians, stakeholders, biologist, disease ecologists/specialists, and social scientist (not normally involved)
- How—participatory approach, pre-designed (not a one day meeting and group hug), rigorous scientific design that might involve questionnaires and surveys
- Goals—to articulate a shared vision of obstacles to consensus, trust building, and collaboration; to establish a burden of proof; to obtain information that assists people in making informed decisions; to discuss study designs that will influence the majority; and to develop measures of success

Part II: A Study of Core Obstacles and Measureable Data

The following questions need to be asked:

- What does a healthy population look like? (locally, regionally, continentally)
- How are we able to achieve the management goals and measure success?
- What are acceptable herd sizes and mortality rates?
 - Establish risks
 - Determine cause and effect studies
 - Determine how to measure effects of change on a population

Once researchers have determined an acceptable rate, they can establish risks.

Question/Answer

After Working Group A's presentation by Dr. Garde, the following issues were discussed:

- When asked how the group suggested determining acceptable mortality, Dr. Garde replied that the group wasn't talking about preventing mortality, but determining the natural mortality that would occur in a herd and the point at which managers would need to step in with preventative measures.
- No consideration to funding or cost was made when developing the ultimate study. Dr. Weiser suggested speaking to politicians for funding since this is a politically-charged issue right now. And, if you want to approach this with a social science aspect, involve the social scientists for additional funding.
- Part I needs to feed into Part II in order to get into the proper framework.

Working Group B

Working Group B was tasked with the objective of identifying and prioritizing field experiments to test hypotheses about the causes of pneumonia in free-ranging bighorn sheep. Working group B was to create a matrix that prioritizes factors associated with pneumonia that are high priorities for future research based on current knowledge and results of previous research and a list of potential manipulative or mensurative experiments in free-ranging, captive, and laboratory conditions that would address the high priority research questions.

Risks that Might be Associated with Respiratory Disease in Sheep

Dr. Garde displayed an Excel spreadsheet that contained a list of risks that might be associated with respiratory disease in sheep (see Appendix D). For each factor, the group had included an argument for and against the factor and had listed what wasn't known about it.

Introduction of novel pathogens

Introductions of novel pathogens have been documented experimentally in captive bighorn sheep and in livestock, but not into free-ranging bighorn sheep populations.

Weather

No experimental evidence exists or has been documented that proves outbreaks are associated with weather.

Overcrowding creating conditions conducive to disease transmission

Heavy densities have been identified as a factor in livestock pneumonia, but no experimental evidence exists. Some large populations of bighorn sheep never experience pneumonia and some are affected only periodically by die offs.

Bottom-up regulation: density-dependence through nutritional constraints

Evidence exists in other wildlife populations and in livestock that poor nutrition can predispose animals to disease, but evidence does not exist for bighorn sheep.

Trace element or other environmental deficiencies

Selenium, copper, and zinc are known to play a role in immune function and trace element levels in forage in some areas used by bighorn sheep are considered marginal or deficient for domestic sheep. However, no experimental evidence exists in free-ranging or captive bighorn sheep.

Anthropogenic stressors such as human disturbance or being held in captivity, and natural stressors such as the rut

Stressors are thought to be important in shipping fever complex in livestock and increased cortisol levels have been shown to be associated with reduced immune function at the cellular level in bighorn sheep. However, no experimental evidence exists for animals.

Ratings

Each of the factors was rated according to the following criteria:

A—wide application across ecosystems/situation

B—scientific merit (does it build on existing knowledge)

C—does it directly affect management decisions

D—does the factor have potentially important population-level effects via either mortality and/or reproduction

E—could this provide the most parsimonious explanation

After each factor was rated, they were listed in the following order of most to least important:

1. Introduction of novel pathogens
2. Nutrition/density
3. Density related disease transmission
4. Natural stressors
5. Anthropogenic stressors
6. Trace elements/other environmental deficiencies
7. Weather

Final Hypotheses:

General hypotheses—organisms that are carried by apparently healthy domestic sheep, but which are lethal to bighorn sheep, are transmitted from domestic sheep to bighorn sheep when these two species commingle.

Sub-hypotheses:

1. When bighorn sheep are exposed to domestic sheep, transmission of novel organisms occurs from domestic sheep to bighorn sheep
2. After transmission, respiratory disease develops in bighorn sheep
3. The organism is transmitted among the bighorn sheep population and causes disease
4. This causes a significant mortality in the population

Experimental design ideas included the following:

1. Replicated experiment (as at Starkey) with tame bighorn sheep. Introduce domestic sheep with marked organisms and monitor health and spread of labeled organisms in tame sheep.
2. Replicated experiment in free-ranging bighorn sheep (10 ecologically similar populations with a five-year control period and then introducing domestic sheep into five of the populations while monitoring the two sets of five populations for another five years) using marked organisms. (This experiment is a perfect example of a BACI [before/after controlled impact] experiment.)

Question/Answer

After Working Group B's presentation by Dr. Cassirer, the following issues were discussed:

- Translocation wasn't included in the list of risk factors because of time constraints, not because members of the group did not think it was important.
- Neither funding nor any other limitations were considered when developing the "ultimate experiment." Politically, experiment number 2 designed by this working group could not be undertaken.
- A replicated marker would have to be used for organisms to retain their markers. A member of the audience has performed a similar experiment in cattle, but cattle infections are self-limiting. However, he believed the organism would move through the population.
- A participant relayed research she had done where the subjects were studied for four years before and after infection. When many areas are researched together, not as many years are needed as one might think.

Question/Answer for Either Working Group A or B

Because of the relationship between the topics addressed in Working Group A and B, a brief period of Q&A for either group was offered. The following issues were discussed:

- The problem seems irresolvable if researchers can't undertake the necessary experiments.
- The experiments described by the working groups would be problematic, but experiment 1 could be undertaken. However, no one could answer what would happen if Part I from Working Group A indicated the necessity of experiment number 2 from Working Group B.
- The ultimate experiment emphasized the need for long-term monitoring data and baseline information. If researchers were diligent and had substantial information prior to perturbations, then data following perturbations could be compared. As much information as possible needs to be collected as often as possible. Secondly, researchers need to ensure they are collecting the correct samples.
- Many good experiments can't be undertaken in the field, but there are plenty of other good experiments that can be done.

Group D:

Ms. Woolever provided opening remarks to Working Group D's presentation. She explained the role of federal land managers, and the social and political realities they are faced with in their daily land and resource management decisions. She provided background information concerning the *Risk Analysis of Disease Transmission between Domestic Sheep and Bighorn Sheep on the Payette National Forest* (Payette RA), which was one of the two risk assessments that Working Group D reviewed.

Working Group D was tasked with four objectives: 1) Provide an overview of disease risks facing wild sheep and their consequences; 2) Define risk, relative risk, and conduct very simple risk calculation exercises; 3) Define very basic risk assessment or analysis and give examples of their limitations and how they may be used in the management of bighorn/domestic sheep; and 4) Consider basic options for management of disease risks facing wild sheep. Desired outcomes for Working Group D included the following: access to historic and foundational literature of disease risks facing wild sheep; recognition of the limitations of current knowledge regarding disease transmission between bighorn and domestic sheep and between different bighorn populations, how much interaction or contact may be required, and difficulties in measuring this; access to basic information on two approaches that have been taken to do risk analysis specific to bighorn/domestic sheep disease; and an appreciation for the complexity and challenges facing managers due to limitations on data, vast time and space, and serious consequences.

The group discussed ways to calculate relative risk, discussed the issues of contact and how to define contact, and moved on to study two particular types of risk assessment: qualitative and quantitative.

Qualitative Risk Assessment—Strengths, Weaknesses, and Application (example: Payette RA)

Strengths:

- Use of broad spectrum of expert resources
- Quick—don't have lots of data, lack of ability to quantify
- Practical—economical
- What you do because you've got to do it
- Allows you to look at risk on a relative basis

Weaknesses:

- Subjectivity
- Can be easily criticized by those not involved
- Potential to erroneously apply to dissimilar areas or situations
- Difficult to update with new information
- Geographic-specific

Application:

- When you lack data/or little data
- Narrow focus—one or two questions it seeks to address
- Using people very familiar with area/situation
 - Where populations are
 - Where problems are
- Geographic-specific

Quantitative Risk Assessment—Strengths, Weaknesses, and Application (example: Sierra Nevada RA)

Strengths:

- Measureable, more defensible
- Modeling methodology transferable
- Can be easily updated with new info
- Helps to answer “what if” management questions, how to minimize risk
- Transparent—easier to track how conclusions made

Weaknesses:

- Need data (but maybe not as much as might think)
- More time and money up front
- Input data may be different or more defined from one area to another

Application:

- If you have good data, do it
- Special status species
- Narrow range of acceptable risk

Dr. Jessup shared how the model used for the Sierra-Nevada RA could display how changes in management would alter the degree of risk.

Workgroup D felt that quantitative RAs were a superior method for conducting RA.

Hybrid Model

The working group discussed whether there was something in-between the qualitative and quantitative analysis that would capitalize on the strengths of both. They made the following recommendations for a "hybrid" analysis:

- Use quantitative for areas/factors that you have data
- Use qualitative for interpretation and analysis of things you cannot measure or don't have data

Risk Assessment Considerations

The group highlighted several considerations for future risk assessments:

- Need to understand both species if you are going to minimize contact
- Template for risk assessment
 - Consistency
 - Template
- Good quality data
- Appropriate model

For example, data for a full year during a year when the bighorn sheep were able to migrate normally would be needed.

Recommendations for the Future

In summary, Working Group D suggested consulting with ecological risk assessment modelers using the Sierra-Nevada model as a starting point to develop a template for decision makers.

Additional recommendations included the following:

- Quantify contact required for disease transmission
- Modeling efforts with other diseases—human or otherwise—lessons learned about transmission
- Comprehensive risk assessment
 - Opportunities to gather data/identification
 - Need to build uncertainty into model
 - Sensitivity analysis
 - Includes ecology and disease

- Inventory of who is working on what and what are they learning?
 - Coordinated function
- Social science analysis and how to bring in this aspect into risk assessment (decisions cannot be made on biology alone)
- Outreach/delivery of risk assessment info (personal touches) and models
- Build model to identify problems; build model on how to minimize risk to make future decisions

The future for ecological risk assessment:

- Includes disease transmission assessment
- Holistic/systems approach
- Gather info on factors to aid in future modeling
- Comparative analysis/studies (need adequate numbers/sample size and populations depending on what is being assessed)
- Be mindful on assumptions being made in modeling

Payette Principles

Dr. Jessup said that Working Group D also discussed the principles which were an outcome of the science panel convened by the Payette National Forest in November 2006 to review and discuss comments received on the Payette RA. Dr. Jessup reviewed the eight principles, and suggested edits from Working Group D, which he displayed onscreen and also provided in handouts to all workshop participants during the presentation (see Appendix H for the principles and suggested edits as supported by Working Group D participants). Workshop participants were asked to review the principles and to state whether they could support them. One workshop participant noted that the principles were important to future efforts, and those who had reservations should voice them.

While Working Group D and a number of other workshop participants supported the eight principles, a workshop participant expressed concern that researchers did not yet completely agree that contact with domestic sheep increases the risk of disease or mortality in bighorn sheep. It was also noted that while it might be prudent to keep bighorn sheep and domestic sheep separated to reduce the risk of disease transmission, this could be accomplished through management, and that separation may not need to be permanent. Another participant said that he wasn't familiar enough with the background related to these principles to endorse them at this time and noted others might also feel that way. Hayman stated that these principles would be brought forward for additional discussion at the risk assessment workshop tentatively scheduled for fall 2007.

Other Question/Answer from Working Group D Presentation

Following Working Group D's presentation by Dr. Jessup, and the discussion of the Payette principles, the following additional issues were discussed:

- To help explain the concept of probability, it was noted there are going to be times when there is a higher probability that bighorn sheep will get sick, but a higher probability doesn't guarantee a disease outbreak. Managers must explain why they aren't saying that every time you put domestic sheep and bighorn sheep together they are going to get sick.
- Another workshop that will be exploring wildlife risk assessment for folks in the biology field is tentatively scheduled for fall of 2007.
- Determining an acceptable risk is based on values. Values, which are a reflection of social science, need to be known and understood when setting limits. Social science plays a significant part, and if researchers and managers aren't talking to each other, parameters may get thrown out because social parameters weren't considered and people who disagree won't want to take part in the study.
- It was acknowledged that social values were not reflected in the Sierra-Nevada quantitative model discussed today, but they were reflected thoroughly throughout the entire assessment process. In

order for domestic sheep to continue grazing in the Sierra-Nevada area, stakeholders need to ensure the bighorn sheep persist. If there is a major die-off of bighorn sheep, the stakeholders would be negatively affected.

- Interested parties can vary greatly and getting consensus can be difficult. Acceptable risk for someone who doesn't want grazing on public lands is zero. When scientists are thinking about research, they need to consider the social aspect, but it can be very difficult and time-consuming to get to consensus.

Funding and collaboration opportunities

Dr. Gonzales asked if Lee could make a comment concerning funding. Lee replied that FNAWS would favor funding priorities concerning disease transmission; interest levels would be lower for esoteric requests.

Dr. Srikumaran spoke about the Wyoming Wildlife/Livestock Disease Partnership. In order to qualify, one of the primary investigators has to be a Wyoming resident. He has secured funds for studying disease transmission over the next two years. However, it is a yearly submission. The funding focuses on three diseases and they tend to fund research on one of the three diseases each year. Ms. Woolever offered to provide the contact information for this particular funding source to anyone who is interested.

Most funders want to see priorities and organization. Once the results of the workshop are posted, and participants see what priorities fall out, they can start talking about funding. Perhaps the momentum of the meeting is a good starting point.

Ms. Woolever stressed scientists need to get together and find out what the knowledge gaps are, how those gaps are going to get filled, and who is going to fill them. Woolever was disappointed that a list of current research and funders had not been compiled; however, work is ongoing. Woolever mentioned that the Western Association of Fish and Wildlife Agencies will be meeting over the next couple of years. It was noted that some working groups did come up with a list of the most productive topics to begin with and a subset of research priorities. A next step may be to create an overall ranking.

Hayman encouraged members to read the meeting summary once it is posted and to look for opportunities to collaborate with other participants to start the important follow up work. Dr. Gonzales added that the results of this meeting will be posted on the internet. His goal is to develop some kind of communication between everyone.

Dr. Cassirer acknowledged that the participants had accomplished a great deal. Not everyone is going to agree and not everyone is going to have the answer, but no one should stop thinking about the solutions after leaving here. The problem wasn't going to be solved in two days and participation doesn't end when everyone walks out the door.

Wrap Up

Dr. Garde concluded the workshop by thanking Alexis Nakamura and Sherri Lee Smith and the Wildlife Health Center for their administrative work; financial supporters, including Steve Torres on behalf of the CDFG, Ray Lee on behalf of FNAWS, and Kyle Meintzer on behalf of California FNAWS; the speakers, Susan Hayman; Dr. Ben Gonzales; and all who took time of their busy schedules to attend.