DISSERTATION

OROPHARYNGEAL BACTERIA, WITH RESPECT TO ANIMAL HEALTH CLASSIFICATION, AND VIRAL SEROLOGY OF MONTANA BIGHORN SHEEP (*OVIS CANADENSIS*) AND DOMESTIC (*OVIS ARIES*) NEAR TO AND DISTANT FROM THE WILDLIFE/DOMESTIC ANIMAL INTERFACE

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DAVID STEVEN MILLER ENTITLED OROPHARYNGEAL BACTERIA, WITH RESPECT TO ANIMAL HEALTH CLASSIFICATION, AND VIRAL SEROLOGY OF MONTANA BIGHORN SHEEP (*OVIS CANADENSIS*) AND DOMESTIC (*OVIS ARIES*) NEAR TO AND DISTANT FROM THE WILDLIFE/DOMESTIC ANIMAL INTERFACE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

OROPHARYNGEAL BACTERIA, WITH RESPECT TO ANIMAL HEALTH CLASSIFICATION, AND VIRAL SEROLOGY OF MONTANA BIGHORN SHEEP (*OVIS CANADENSIS*) AND DOMESTIC (*OVIS ARIES*) NEAR TO AND DISTANT FROM THE WILDLIFE/DOMESTIC ANIMAL INTERFACE

Respiratory disease outbreaks attributed to pasteurellosis have lead to conflict at the wildlife/domestic interface, where domestic sheep have been hypothesized to be a reservoir of Pasteuerellaceae strains that cause disease in bighorn sheep. This dissertation compares bighorn sheep (*Ovis canadensis*) and domestic sheep (*O. aries*) oropharyngeal Pasteurellaceae biovariants from animals classified as diseased and healthy. It also compares bacteriology and viral serology of populations of these species near to and distant from the wildlife/domestic livestock interface. A retrospective study of clinical submissions (1990 – 2004) indicated that 94 Pasteurellaceae biovariants have been associated with domestic sheep classified as diseased. A second retrospective study (1989 – 2004) indicated that 37 Pasteurellaceae biovariants have been associated with bighorn sheep classified as diseased. A prospective study of domestic and bighorn sheep near to and distant from the wildlife/domestic interface indicated that Pasteurellaceae biovariants commonly associated with disease in the retrospective studies were also common in healthy animals, and that there was extensive interspecific sharing of biovariants. This

suggests that a simple agent/disease relationship may not exist for Pasteurellaceae in these host species. In addition, it is not clear that either species serves as a reservoir for Pasteurellaceae that are pathogenic for the sympatric species. However, unstated assumptions that single samples represent an animal's Pasteurellaceae microflora are questionable, based on the minimal concordance of biovariants of individual domestic livestock (n = 118) sampled six months apart. Based on the populations in the prospective study, bighorn sheep populations were naïve to *Mycoplasma*, and both *Ovis* species were largely naïve to infectious bovine rhinotracheitis and bovine virus diarrhea 1 and 2. This suggests that these agents may cause outbreaks if introduced into these populations. Cluster analysis of Pasteurellaceae and viral serology results identified four different clusters ($P < 0.0001$), but these did not closely correspond to species and location categories. The results from this study suggest that emphasis on single determinants for causes of respiratory disease outbreaks in domestic and bighorn sheep, rather than determination of risk factors for multiple determinants, may not provide results that are useful for managing disease in these species.

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CHAPTER 1

INTRODUCTION

This chapter summarizes this dissertation's structure. The aim of this dissertation is to evaluate bighorn (*Ovis canadensis*) and domestic sheep (*O. aries*) for evidence of shared agents with presumed potential for causing respiratory disease at and > 14.5 km from the wildlife/domestic livestock interface. The objectives are:

1. Identification of Pasteurellaceae associated with bighorn and domestic sheep with and without apparent respiratory disease.
2. Identification of shared Pasteurellaceae from bighorn and domestic sheep without apparent respiratory disease in populations located at and > 14.5 km from the wildlife/domestic livestock interface.
3. Survey bighorn and domestic sheep populations without apparent respiratory disease located distant to and at the wildlife/domestic livestock interface for evidence of shared infections with *Mycoplasma* spp., parainfluenza-3 (PI-3), bovine respiratory syncitial virus (BRSV), infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD) 1 and 2, and fecal parasites.

This research project was conceived due to a need to explore new approaches for understanding and resolving the cause of respiratory disease outbreaks in bighorn sheep, as well as the potential for respiratory agent transmission at the bighorn/domestic sheep interface. Secondary objectives included in this dissertation are animal and population characteristics that may be explored more fully in subsequent studies for their role in predicting or managing disease in bighorn and domestic sheep.

Chapter 2 is a literature review that summarizes information on respiratory disease in bighorn and domestic sheep. It is provided as background for the importance and justification for the research conducted for this dissertation.

Chapter 3 reports on Pasteurellaceae isolates from bighorn sheep clinical submissions to the Caine Veterinary Teaching Hospital 1989 – 2004. Submissions were associated with animals characterized as having respiratory disease or apparently healthy. Many submissions did not associate samples with a specific animal. Consequently, the most relevant data in this chapter is a list of biovariants associated with animals characterized as having respiratory disease.

Chapter 4 reports on Pasteurellaceae isolates from domestic sheep clinical submissions to the Caine Veterinary Teaching Hospital 1990 – 2004. Submissions were associated with animals characterized as having respiratory disease or apparently healthy. Most submissions did not associate samples with a specific animal. Consequently, the most relevant data in this chapter is a list of biovariants associated with animals characterized as having respiratory disease.

Chapter 5 reports the results of a questionnaire administered to domestic sheep and goat producers. This was based on United States Department of Agriculture, National Animal Health Monitoring System questionnaires, and was conducted as a pilot study for information on domestic sheep operations. It provides baseline information on population sizes, management, potential interspecies agent transmission, and producer attitudes towards bighorn sheep that were hypothesized to be potentially useful for developing management strategies for resolving bighorn-domestic sheep conflicts.

Chapter 6 is a cross-sectional study of bighorn and domestic sheep populations distant to and at the wildlife/domestic livestock interface. This study provides baseline Pasteurellaceae data on animals that are largely without apparent clinical abnormalities. This allowed qualitative comparisons of Pasteurellaceae isolates from each host species

with respect to location at or distant to the wildlife/domestic livestock interface. It also permitted qualitative comparisons with isolates from animals classified as having respiratory disease in chapters 3 and 4. Assumptions that single sample events are representative of an animal's oropharyngeal Pasteurellaceae were evaluated by resampling individual domestic livestock twice, six months apart. As the role of other agents in the development of respiratory disease is unclear, samples were also concurrently collected for *Mycoplasma* spp., and viral serology for PI-3, BRSV, IBR, BVD 1, and BVD-2. Results from assays for these agents identified populations that were naïve to these agents and were incorporated into a cluster analysis conducted to identify assemblages of agents that were characteristic of species and locations relative to the wildlife/domestic livestock interface.

Chapter 7 critiques the study design of the dissertation and discusses possible future directions for research on bighorn and domestic sheep respiratory disease.

The conceptual hypothesis of this dissertation is that if cross-species transmission of a single agent is responsible for causing respiratory disease at the bighorn/domestic sheep interface as a primary pathogen, the agent must be consistently associated more commonly with diseased animals, the agent may be present without apparent disease in source or reservoir species, and that the impact of other agents should be minimal. As the available data limit direct assessment of this hypothesis, the operational hypotheses are:

1. Ho_1: biovariants commonly associated with respiratory disease are also commonly associated with healthy animals.
2. Ho_2: the oropharyngeal Pasteurellaceae biovariants of an individual will be similar with repeated sampling.

3. Ho_3: the assemblages of Pasteurellaceae biovariants, *Mycoplasma* spp., parainfluenza-3, bovine respiratory syncitial virus, bovine virus diarrhea 1 and 2, infectious bovine rhinotracheitis in bighorn and domestic sheep populations are similar.

The alternate operational hypotheses are that there are Pasteurellaceae biovariants that are most commonly associated with animals with respiratory disease, that there is temporal variation in an individual's oropharyngeal Pasteurellaceae, and that there are agents that are primarily associated with a single host species.

This dissertation addresses the need for comparisons of infectious agents in multiple bighorn and domestic sheep populations, and also establishes baseline data on healthy animals. These comparisons are important for placing findings from animals with respiratory disease in the appropriate context. This dissertation largely utilizes qualitative methods as a basis for establishing context and as a provisional means of understanding the agents associated with respiratory disease in these species. This is analogous to the use of qualitative data for development of theory in the health sciences (Bradley *et al.*, 2007; Fletcher *et al.*, 2009; Neergaard *et al.*, 2009).

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CHAPTER 2

LITERATURE REVIEW

Background

Bighorn sheep (*Ovis canadensis*) are a high profile species that were historically widespread over a range of arid and mountain habitats in western North America, and have long been important to humans as a source of food, as well as for spiritual and aesthetic reasons (Toweill & Geist, 1999). However, die-offs due to outbreaks of respiratory disease and other causes have substantially reduced free-ranging bighorn numbers and range for over a century (Baillie-Grohman, 1902; Buechner, 1960). These die-offs have been associated with settlement of western North America (Valdez & Krausman, 1999). Although many resources have been expended to reintroduce bighorn sheep to historic range and increase population sizes (Toweill & Geist, 1999), reestablishment of stable, self-sustaining bighorn sheep populations has sometimes been hindered by disease outbreaks (Gross *et al.*, 2000; Singer *et al.*, 2001). Pasteurellosis is currently considered a principle cause of respiratory disease outbreaks in bighorn sheep (Council for Agricultural Science and Technology (CAST), 2008).

The introduction of domestic sheep (*Ovis aries*) into historic bighorn sheep range corresponds with the decline of bighorn sheep numbers (Buechner, 1960). Subsequently, the domestic sheep industry declined substantially over the last half century due to multiple causes (National Research Council, 2008). Restrictions on domestic sheep grazing allotments on public lands (United States Geologic Survey/Bureau of Reclamation Office, 2006) where bighorn sheep exist or can be reintroduced pose limitations on domestic sheep industry recovery efforts in some locations. In addition, there is potential for conflict where domestic sheep are used for exotic weed control (Olson & Lacey, 1994) and other activities where bighorn sheep are present or could be reintroduced.

Consequently, there is tension over land use between domestic sheep and bighorn sheep recovery efforts.

Disease outbreaks and die-offs

Disease outbreaks are a shared concern for bighorn and domestic sheep. Disease outbreaks are defined as increases in disease or death beyond typical levels (Martin *et al.*, 1987). Domestic sheep losses are more easily defined and recognized, due to their proximity to humans, and more easily quantified in financial terms. For bighorn sheep, "outbreak" is a more subjective term, as baseline levels of morbidity and mortality are generally unknown for most populations, and outbreaks are generally recognized subjectively and fortuitously. Increases in mortality (die-offs) are the ultimate concern for bighorn sheep outbreaks when they result in marked reductions in population sizes. Similar concerns exist for domestic sheep and other livestock industries when respiratory disease outbreaks compromise herd health (Watson & Davies, 2002; Cusack *et al.*, 2003).

Bighorn sheep die-offs

Bighorn sheep population declines were initially associated with overhunting and overgrazing that accompanied settlement of western rangelands. Unregulated hunting in the 1800s and early 1900s substantially reduced or eliminated many bighorn sheep populations (Buechner, 1960). Concurrently, die-offs occurred due to starvation caused by livestock overgrazing. These die-offs are believed to be distinct and additive to hunting (Bailey, 1936; Davis & Taylor, 1939; Marsh, 1938; Packard, 1946).

Disease related die-offs in bighorn sheep were recognized shortly after settlement of western rangelands. Multiple determinants have been proposed over the past century to explain these die-offs (Potts, 1938; McCann, 1956; Bunch *et al.*, 1999). First recognized were scabies (*Psoroptes* spp.) outbreaks, which were novel events that had not previously been recognized by native Americans (Hornaday, 1901; Baillie-Grohman, 1902; Grinnell, 1904; Buechner, 1960). Subsequently, in the middle 20th century, lungworm (*Protostrongylus* spp.), was the primary agent associated with bighorn sheep die-offs (Pillmore, 1958b). Pasteurellaceae are currently believed to be the agents primarily responsible for bighorn sheep die-offs, due to isolation of these organisms from bighorn sheep with respiratory disease (Council for Agricultural Science and Technology (CAST), 2008). Because Pasteurellaceae also appear to be a part of normal, endogenous bighorn sheep oropharyngeal microflora (Miller, 2001), it has also been hypothesized that respiratory disease outbreaks in bighorn sheep may be the consequence of exposure to stressors which cause immunosuppression, thereby increasing susceptibility to disease (Spraker *et al.*, 1984).

Early reports of pasteuerellosis associated with bighorn sheep mortality suggested that *Pasteurella* spp. (which consisted of the current genera *Pasteurella, Mannheimia,* and *Bibersteinia*; herein listed as *Pasteurella* unless otherwise distinguished) were opportunistic pathogens (Potts, 1937; Marsh, 1938). Isolation of *Pasteurella* in pure culture from pneumonic bighorn sheep in a captive population suggested that *Pasteurella* could be primary pathogens (Post, 1962). Subsequent captive bighorn sheep/domestic sheep exposure trials and experimental inoculation research, prompted by a bighorn sheep die-off in Canada, suggested that domestic sheep could be clinically asymptomatic

reservoirs for *Pasteurella* that are pathogenic to bighorn sheep (Onderka *et al.*, 1988; Onderka & Wishart, 1988). This hypothesis was reinforced by subsequent mixed species captive pen studies and evidence, on a molecular basis, for a species specific susceptibility of bighorn sheep to pasteurellosis (Foreyt, 1989; Silflow & Foreyt, 1994; Foreyt & Lagerquist, 1996; Kraabel & Miller, 1997; Dassanayake *et al.*, 2008). Furthermore, isolates of *Pasteurella* from free-ranging bighorn sheep during die-offs has been associated with sympatric domestic sheep and goats (*Capra hircus*) (Rudolph *et al.*, 2003; George *et al.*, 2008). However, as baseline data were not available for comparison, it was not possible to determine whether the *Pasteurella* were primary pathogens responsible for the die-offs. The absence of baseline data also precluded establishment of whether transmission occurred and if so, its direction.

Inferences from post-mortem bighorn sheep outbreak data are limited because without baseline data, it is not possible to distinguish between *Pasteurella* that are present in apparently healthy bighorn sheep and those associated with clinical disease. Antemortem data from sympatric bighorn and domestic sheep populations on four bighorn sheep ranges in Nevada suggested that healthy animals of both species could share *Pasteurella* (Ward *et al.*, 1997). However, confidence in this conclusion is limited by the disappearance of two bighorn sheep populations of undetermined cause during the study. More recently, *Pasteurella* appeared to be shared among two California bighorn sheep populations and domestic sheep using both conventional biogroup and more recently developed biovariant classification schemes (Tomassini *et al.*, 2009). This study assessed *Pasteurella* at both large (biogroup) and fine (biovariant) scales, as the biovariant scheme was developed due to untypable isolates from wildlife and distinguishes among many

more strains than the biogroup scheme is capable of (Jaworski *et al.*, 1998). Because limited baseline data is available for distinguishing among apathogenic and potentially pathogenic *Pasteurella*, and because multiple parasitic, bacterial, and viral agents have also been isolated from free-ranging bighorn sheep with respiratory disease (Marsh, 1938; Pillmore, 1958a; Aune *et al.*, 1998; Rudolph *et al.*, 2007; Besser *et al.*, 2008), it is currently uncertain as to whether and to what magnitude *Pasteurella* is responsible for bighorn sheep die-offs.

Domestic sheep pasteurellosis

While the catalyst for this dissertation research project was the belief that domestic sheep may serve as apparently healthy reservoirs for *Pasteurella* that are pathogenic to bighorn sheep, pasteurellosis is also of direct concern to the productivity of domestic sheep operations. Among the more important production losses to the domestic sheep industry are those due to respiratory disease, with pasteurellosis being one of the more important causes of respiratory disease (Pugh, 2002; USDA, 2005; USDA, 2007). There have been reports suggesting that pasteurellosis is a primary infectious disease in domestic sheep outbreaks (Mishra *et al.*, 2000; Watson & Davies, 2002). However, the commonly accepted ruminant model of pasteurellosis ("shipping fever") considers various combinations of host, agent, and environmental factors as predisposing causes of pasteurellosis (Brogden *et al.*, 1998; Ackermann & Brogden, 2000). In accord with this model, there are reports supporting pasteurellosis as a secondary pathogen in domestic sheep (Odugbo *et al.*, 2004; Shiferaw *et al.*, 2006; Lacasta *et al.*, 2008).

Pasteurella are considered opportunistic pathogens in shipping fever (Ackermann & Brogden, 2000). Consequently, pneumonic pasteurellosis develops when some combinations of host, agent, and environmental determinants favor pulmonary colonization by endogenous oropharyngeal *Pasteurella* (Yates, 1982; Czuprynski *et al.*, 2004; Zecchinon *et al.*, 2005; Dabo *et al.*, 2008). A corollary is that management that minimizes the determinants favoring pulmonary colonization may minimize the odds of disease development. Recognition that *Pasteurella* are a normal part of animal's oropharyngeal microflora, in combination with a lack of concordance among experiments that pursued single agent hypotheses, were the concepts that shaped this model of pasteurellosis. The shipping fever model and the scientific process behind the development of this model may be relevant to bighorn sheep pasteurellosis in terms of appropriate models for the biology of this disease, as well as logical corollaries for developing potential management strategies.

Pasteurellaceae classification

Mannheimia haemolytica, Pasteurella (Bibersteinia) trehalosi, and Pasteurella multocida have undergone multiple taxonomic changes (Table 2.1) (Biberstein *et al.*, 1991b; Jaworski *et al.*, 1998; Miller, 2001). Several methods of subclassifying P/M have been used. *Pasteurella multocida* is conventionally classified by five capsular serogroups and 16 somatic serotypes (Confer, 1993). Subspecies and biotypes have also been identified biochemically (Biberstein *et al.*, 1991a). *Mannheimia haemolytica* and *B. trehalosi* have also conventionally been classified by capsular antigens into serotypes (Confer, 1993; Blackall *et al.*, 2007). However, cross-agglutination or non-reactions with

typing sera prevent classification of many isolates from bighorn sheep. Consequently, a biovariant classification scheme based on microbiological characteristics and biochemical utilization tests of isolates in culture was established to minimize the number of isolates which cannot be assigned to serotypes (Bisgaard & Mutters, 1986; Jaworski *et al.*, 1998). The biovariant classification scheme is hierarchical by species, type, and exceptions, with species being the broadest category, type being a subcategory of species, and exceptions being the most specific category (biovariant). The biovariant level of classification distinguishes among a greater number of P/M strains than do serotype classification schemes. Biovariants and biogroups are not directly comparable (Table 2.1).

The bighorn/domestic sheep interface

There is a long history of conflict over land use between domestic animal interests and wildlife (Conover & Conover, 1997). More recently, recognition of pathogen transmission at the wildlife/domestic animal interface has become a concern (Gibbs & Bokma, 2002; Osofsky *et al.*, 2005). Potential agent exchange between bighorn and domestic sheep may be considered in the broader context of the historical conflict over land use and pathogen transmission at the wildlife-domestic animal interface. Transmission of agents from wildlife reservoirs to domestic animals is a concern for companion animals and livestock productivity, particularly for agents of regulatory concerns. Although transmission of agents from domestic animals to wildlife often receives less attention, this concern has led to land use policies for keeping bighorn and domestic sheep separate by 14.5 km (Unites States Department of the Interior, 1998). This

policy contributes to competition for land, and this conflict is likely to intensify as land development in the western states continues. While this conflict in large part reflects social values for land use, biological concerns for pathogen transmission exist and are a basis for debate when land use policy is considered (United States Geologic Survey/Bureau of Reclamation Office, 2006).

Conclusion

Resolution of the biological debate on the role of pasteurellosis in bighorn sheep outbreaks is dependent upon resolving whether *Pasteurella* can act as primary pathogens that are responsible for die-offs, as well as for identification of *Pasteurella* reservoirs. Similar information is needed for pasteurellosis in domestic sheep. Conversely, it is important to identify instances where pasteurellosis represents opportunistic or incidental infections, as management strategies under these scenarios may be best directed at the primary determinants, rather than the agent(s). However, without more extensive baseline data, it is difficult to address these uncertainties. This dissertation will utilize retrospective data to identify potential pathogenic biovariants. It will also utilize a cross-sectional study to clarify which *Pasteurella* and other potential respiratory disease agents are present in apparently healthy bighorn and domestic sheep, at and distant to their interface. As respiratory disease outbreaks are sporadic and unpredictable, and as only cross-sectional data was available for this dissertation research project, this dissertation will focus on baseline identifications of shared pathogens, relative to host species and apparent animal health status. This data will provide perspective to future studies concerned with agents responsible for outbreaks of respiratory disease in bighorn and domestic sheep.

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Table 2.1. Species and biovariants of Pasteurellaceae with respect to previous nomenclature and serotypes (Biberstein *et al.*, 1991b; Jaworski *et al.*, 1998; Miller, 2001).

Current nomenclature	Previous nomenclature	Biogroups (Serotypes)	Biovariants
Mannheimia haemolytica	*Pasteurella haemolytica* *Pasteurella ovisepticum*	1, 2, 5-9, 11-14, 16	1, 3, 5-10, 16 and U
Pasteurella multocida	*Pasteurella multocida*	Capsular: A, B, D, E, F Somatic: 1 -16 Capsular: B, E Somatic: 2	Various species and biotypes
Bibersteinia trehalosi	*Pasteurella trehalosi* *Pateurella haemolytica* biotype T	3, 4, 10, 15	2 and 4

CHAPTER 3

BIGHORN SHEEP PASTEURELLACEAE ISOLATES FROM SUBMISSIONS TO THE CAINE VETERINARY TEACHING CENTER (1989-2004)

Abstract

This study was conducted to identify Pasteurellaceae that were isolated from bighorn sheep (*Ovis canadensis*) with respiratory disease, based on diagnostic samples submitted to a reference laboratory (Caine Veterinary Teaching Center) from 1989 – 2004. Submissions generally consisted of nasal or oropharyngeal samples from multiple animals, but submission information generally precluded associating samples or bacterial isolates with specific animals. Zero to multiple bacterial isolates were obtained from samples. Isolates (n = 767) were composed of four species of Pasteurellaceae: *Haemophilus somnus, Mannheimia haemolytica, Pasteurella multocida,* and *Pasteurella* (*Bibersteinia*) *trehalosi*. Among the latter three species, 115 biovariants were identified. Biovariants were identified 1 – 246 times. Most isolates were from adults (n = 675), and most (97%) of these were from animals without apparent clinical abnormalities. In contrast, isolates from juveniles (n = 92) were generally (89%)) associated with animals with signs of respiratory disease. Twenty-two biovariants were associated with animals classified as having respiratory disease, and these comprised 14% of the total number of isolates. With the exception of *M. haemolytica* $16^{\alpha E}$ (n = 1 isolate), biovariants were isolated more often from adult bighorn sheep without signs of disease than from adults with signs of respiratory disease. In contrast, biovariants isolated from juveniles were more often associated with animals with signs of respiratory disease, with the exception of *M. haemolytica* $10^{\alpha B}$ (n = 3 isolates). With the exception of three biovariants (*M. haemolytica* 9^{B}, *P.* (*B.*) *trehalosi* 2^{BG}, and *P.* (*B.*) *trehalosi* ^{2CDS}) which accounted for a total of 4 isolates, each of the biovariants isolated from clinically diseased juveniles was isolated from apparently healthy adults. There were no differences detected among animals based

on health (respiratory disease or apparently healthy) when isolates were evaluated at higher, species (P = 0.60) or type (P = 0.16) taxonomic levels. There was an association between isolate beta-hemolysis and animals with respiratory disease (P < 0.0001; OR 2.73, 95% CI 1.78 – 4.14). While the inference of this study is limited, it provides a baseline list of biovariants that are associated with disease in domestic sheep.

Key words: bighorn sheep, *Ovis canadensis* *Mannheimia* (*Pasteurella*) *haemolytica, Pasteurella* (*Bibersteinia*) *trehalosi*, *Pasteurella multocida,* respiratory disease

Introduction

Pasteurellosis is considered a significant risk for respiratory disease and mortality in bighorn sheep (Bunch *et al.*, 1999; Miller, 2001). Mortalities due to Pasteurellaceae pneumonia are considered a limiting factor for bighorn sheep populations, as is depressed fecundity that can occur subsequent to respiratory disease die-offs (Gross *et al.*, 2000; Cassirer & Sinclair, 2007; George *et al.*, 2008). Pasteurellaceae species commonly associated with respiratory disease epidemics in bighorn sheep are *Mannheimia* (*Pasteuerella*) *haemolytica* (Angen *et al.*, 1999), *P.* (*Bibersteinia*) *trehalosi* (formerly *P. haemolytica* biotype T)(Sneath & Stevens, 1990; Blackall *et al.*, 2007), or *Pasteurella multocida* (Miller, 2001; Weiser *et al.*, 2003; George *et al.*, 2008). Although *Pasteurella* and *Mannheimia* spp. (P/M) isolates from bighorn sheep have long been reported with other potential pathogens and as opportunistic pathogens (Evans, 1937; Marsh, 1938), there have also been isolates in pure culture from captive bighorn sheep during an outbreak (Post, 1962). In domestic animal models of pasteurellosis, disease from P/M is

considered the consequence of interactions of host, environment, and agent determinants (Yates, 1982; Czuprynski *et al.*, 2004; Zecchinon *et al.*, 2005; Dabo *et al.*, 2007). In contrast, P/M has been hypothesized to be a primary pathogen in bighorn sheep, with domestic sheep as a possible reservoir (Onderka *et al.*, 1988; Foreyt *et al.*, 1994; Dassanayake *et al.*, 2009). Consequently, there has been conflict over land use policies where there is potential for bighorn and domestic sheep interactions (Council for Agricultural Science and Technology (CAST), 2008).

Several methods of classifying P/M exist. *Pasteurella multocida* is conventionally classified by five capsular serogroups and 16 somatic serotypes (Confer, 1993). Subspecies and biotypes have also been identified biochemically (Biberstein *et al.*, 1991). *Mannheimia haemolytica* and *B. trehalosi* have also conventionally been classified by capsular antigens into serotypes (Confer, 1993; Blackall *et al.*, 2007). However, cross-agglutination or non-reactions with typing sera prevent classification of many isolates from bighorn sheep. Consequently, a biovariant classification scheme based on microbiological characteristics and biochemical utilization tests of isolates in culture was established to minimize the number of isolates which cannot be assigned to serotypes (Bisgaard & Mutters, 1986; Jaworski *et al.*, 1998). The biovariant classification scheme is hierarchical by species, type, and exceptions, with species being the broadest category, type being a subcategory of species, and exceptions being the most specific category (biovariant). The biovariant level of classification distinguishes among a greater number of P/M strains than do serotype classification schemes.

Limited data are available regarding P/M biovariants of bighorn sheep with respiratory disease (Jaworski *et al.*, 1998; Weiser *et al.*, 2003; Rudolph *et al.*, 2007).

Consequently, the aim of this chapter is to identify P/M biovariants associated with bighorn sheep classified as having respiratory disease. This chapter is a study of the P/M biovariants isolated from bighorn sheep clinical samples submitted to a reference laboratory from 1989 - 2004.

Methods

Bacterial samples from free-ranging bighorn sheep submitted to the Caine Veterinary Teaching Center (CVTC) from January 1, 1989 – December 31, 2004 were included in this study, except for isolates from a 1995-1996 outbreak in Hells Canyon that were previously reported (Rudolph *et al.*, 2003; Weiser *et al.*, 2003; Rudolph *et al.*, 2007). Oropharyngeal and nasal swab samples that were placed in varying brands of commercial transport media for bacterial culture were submitted to CVTC by wildlife biologists and veterinarians during the course of bighorn sheep research or management activities. Submissions generally consisted of multiple samples from multiple animals. Because submission information generally prevented associating samples with a specific animal or anatomical location, bacteriology results are reported only on an isolate basis. Each sample yielded zero to multiple bacterial isolates. Isolates described in this report were those which included more complete submission information, e. g., the date of submission, geographic location, health classification (without clinical abnormalities or with signs of respiratory disease, hereafter referred to as healthy or diseased, respectively), and age class (adult or juvenile). Results were from a minimum of 80 different animals, based on the number of submissions; it is not possible to determine the total number of animals that were actually sampled.

Bacterial culture procedures

Samples were shipped overnight on cold packs and plated within 72 hours of collection. At CVTC the samples were inoculated onto nonselective Columbia blood agar (CBA), (Becton Dickinson & Co., Sparks, Maryland 21152, USA) containing 5% sheep blood, and selective Columbia blood agar with selective antibiotics, containing 5% bovine blood (Jaworski *et al.*, 1993), and incubated for 18 to 24 hr at 37°C in a 10% CO_2 atmosphere. Following incubation, representatives of each colony type were propagated on fresh CBA for species and biovariant classification.

Species and biovariant classification of bacterial isolates

Isolates were determined to be *M. haemolytica* or *P.* (*B*). *trehalosi*, as opposed to *P. multocida*, based on the following characteristics: urea- and indole-negative; oxidase-, nitrate-, glucose-, sucrose, and mannitol-positive; and failure to grow or poor growth on MacConkey's agar. *Mannheimia haemolytica* and *P.* (*B*). *trehalosi* were further distinguished if they were trehalose-negative-or trehalose-positive, respectively. Biovariant classification was done using a modification of a biochemical testing system developed for isolates from domestic animals (Bisgaard and Mutters, 1986), adapted for identifying isolates from wildlife (Jaworskiet al., 1998). Briefly, the wildlife method uses the results from 23 microbiological characteristics and biochemical utilization tests to separate isolates into biovariants which are hierarchically classified based on species, type, and exceptions. In addition, isolates with zones of hemolysis on blood agar were classified

as beta-hemolytic. Following speciation and biovariant classification, isolates were stored frozen at -70 C in phosphate-buffered saline: glycerol (4:6 v/v, pH 7.2).

Statistics

Data from submission sheets were entered into a Microsoft Access database (Microsoft Corporation, One Microsoft Way, Redmond WA 98052 USA) at the CVTC and subsequently imported into an Access database developed for this study. For this study, accuracy was confirmed and corrections made by examining original laboratory logs and submissions. Descriptive data and tables were developed directly from the database or after export into Microsoft Excel spreadsheet files.

Statistical analysis was conducted using data exported from the study database into SAS 9.2 (SAS Institute, Inc., Cary, NC 27513 USA). Exploratory analyses were conducted using the FREQ Procedure: chi-square analyses were considered to be significant at $P < 0.05$, and odds ratios were calculated for 2 X 2 tables. Fisher's exact test (n = 100,000 simulations) was used in place of chi-square analyses where there were multiple cells with expected values less than 5. Chi-square and Fisher's exact tests assume independence for data. Separate analyses were conducted for biovariants at each taxonomic level (species, type, and biovariant) to determine whether there was an association with the host animal's apparent health status. Chi-square analysis of biovariants was conducted to determine whether there was an association between the host animal's health classification and whether the isolate was beta-hemolyitic.

Results

Isolates (n = 767) were composed of four species of Pasteurellaceae: *Haemophilus somnus* (n = 2), *Mannheimia haemolytica* (n = 270), *Pasteurella multocida* (n = 35), and *Pasteurella* (*Bibersteinia*) *trehalosi* (n = 452), as well as eight isolates that could not be identified to species (Table 3.1). Among the latter three Pasteurellaceae species, 115 biovariants were identified (Table 3.1). The maximum number of times a single biovariant was isolated was 246 (32% of isolates) for *P.* (*B.*) *trehalosi* 2 (Table 3.2).

Ten isolates were from Wyoming bighorn sheep, 45 from Oregon, and the remainder were from Idaho (n = 712). Over one hundred samples were submitted from bighorn sheep in 1991, 1997, and 1999; there were no submissions for 1995 and 1996 (Table 3.3). As only four years (1989, 1990, 1991, and 2001) had >5 values for submissions classified as from diseased or healthy animals, statistical analyses based on year were not attempted. There was substantial yearly variation in the biovariants identified (Table 3.4).

Most isolates were from adults (n = 675), and most (97%) of these were from animals without apparent clinical abnormalities (Table 3.1). In contrast, isolates from juveniles (n = 92) were generally (89%)) associated with animals with signs of respiratory disease. Twenty-two biovariants were associated with animals classified as having respiratory disease, and these comprised 14% of the total number of isolates. With the exception of *M. haemolytica* $16^{\alpha E}$ (n = 1 isolate), biovariants were isolated more often from adult bighorn sheep without signs of disease than from adults with signs of respiratory disease. In contrast, biovariants isolated from juveniles were more often associated with animals with signs of respiratory disease, with the exception of *M.*

haemolytica $10^{\alpha B}$ (n = 3 isolates)(Table 3.5). With the exception of three biovariants (*M. haemolytica* 9^{B}, *P.* (*B.*) *trehalosi* 2^{BG} , and *P.* (*B.*) *trehalosi* ^{2CDS}) which accounted for a total of 4 isolates, each of the biovariants isolated from clinically diseased juveniles was also isolated from apparently healthy adults.

Evaluation of data at the species taxonomic level identified *P.* (*B.*) *trehalosi* (59%), *M. haemolytica* (35%), and *P. multocida* (5%) as the most common species, with *P.* (*B.*) *trehalosi* having the highest percentage (14%) of samples from sheep classified as diseased (Table 3.2). There was no significant difference (P = 0.60) among these bacterial species by host animal disease classification. Forty-five isolates (6% of isolates) that were not identified to species or which had <5 isolates for an animal health classification were not included in the analysis.

Evaluation of data at the type taxonomic level identified *P.* (*B.*) *trehalosi* 2 (55%), *M. haemolytica* 3 (7%), and *M. haemolytica* 1 (6%) as the three most common isolates, with *M. haemolytica* 9 having the highest percentage (23%) of samples from animals classified as diseased (Table 3.2). There was no significant difference (P = 0.16), using the Fisher's exact test, among these isolates by host animal disease classification. Fisher's exact simulations were based on a sample size of 757 isolates.

Evaluation of data at the biovariant (exception) level identified *P.* (*B.*) *trehalosi* 2 (32%), *P.* (*B.*) *trehalosi* 2^{B} (19%), and *M. haemolytica* 3 (2.5%) as the most common isolates, with *M. haemolytica* 3 having the highest percentage (53%) of samples from animals classified as diseased (Table 3.2). No analysis was conducted for biovariants based on health classification because only three biovariants (*P.* (*B.*) *trehalosi* 2, *P.* (*B.*)

trehalosi 2^{B}, and *M. haemolytica* 3), consisting of 53% of the data, had >5 values in both animal health classification cells.

The odds of an isolate from an animal with respiratory disease being beta hemolytic were estimated to be 2.73 ($P < 0.0001$; 95% CI 1.78 – 4.14) times the odds of an isolate from bighorn sheep without apparent disease being beta hemolytic.

Discussion

This data set is a comprehensive list of Pasteurellaceae biovariants isolated from bighorn sheep diagnostic samples submitted to the CVTC (Table 3.1). The minority of adult (3%) and the majority of lamb (89%) isolates were associated with animals that were clinically diseased, and isolates could not be associated with individuals. This was a retrospective study of clinical submissions where swab collection methods, swab type, animal health classification, and transport media were not standardized. In addition, most samples were from Idaho, submitted in 1994 (Table 3.3), and there was substantial yearly variation in the biovariants present (Tables 3.3 and 3.4). Consequently, it is unlikely that the assumptions of random samples, independent observations, and similar distributions of data in comparisons were met for statistical analyses. Therefore, although laboratory protocols were consistent and it is assumed that bacterial classifications are stable, caution is warranted on the degree of inference possible from these results. However, this data is of value for a preliminary assessment of Pasteurellaceae strains associated with disease in bighorn sheep.

Although most of the isolates in this study were associated with animals classified as apparently healthy, 22 different biovariants were associated with animals classified as diseased. *Mannheimia haemolytica, P. multocida*, and *P.* (*B.*) *trehalosi* (formerly *P. haemolytica* biotype T) have previously been associated with disease in bighorn sheep (Onderka *et al.*, 1988; Weiser *et al.*, 2003; Rudolph *et al.*, 2007; George *et al.*, 2008). These taxonomic categories of bacterial isolates are often used in diagnoses of respiratory disease. However, they actually represent an assemblage of bacterial lineages that may not have similar levels of pathogencity.

Although it is presumed that narrower taxonomic or molecular classification schemes may be more useful for disease investigations, this has not been established for Pasteurellaceae in bighorn sheep. As a preliminary means of addressing this, isolates were evaluated at each taxonomic level (species, type, exceptions)(Table 3.2). At all three classification levels, *P.* (*B.*) *trehalosi* were the most numerous, followed by *M. haemolytica*, although no statistical associations with animal health classifications were identified. In contrast, the percentage of isolates associated with animals with clinical disease was higher for *M. haemolytica* 3 (53%) and *M. haemolytica* 1 (22%) than for *P.* (*B.*) *trehalosi* 2 (17%). This suggests that the most numerically common isolates may not be those that are most commonly associated with disease, although it is difficult to determine this without baseline information on the populations from which these samples were collected (the denominator). It also is consistent with assumptions that the fine scale resolution associated with biovariant classifications schemes may be required to accurately identify Pasteurellaceae lineages that are most commonly associated with disease. However, epidemiological data is required to identify which biovariants have the

greatest impact on natural populations, as population level effects are the consequence of pathogenicity, transmission, risk of exposure, and other factors. It is notable that there is a large gap in prevalence between the most common biovariants in this study, and the biovariants that were less commonly identified; *P.* (*B.*) *trehalosi* 2 and *P.* (*B.*) *trehalosi* 2^{B} account for 51% of the isolates, and none of the other 113 biovariants identified accounted for > 3 % of the total (Table 3.2).

The data available for this study did not support quantitative estimates for identifying P/M that were most commonly associated with respiratory disease. Consequently, as a preliminary assessment, biovariants can be qualitatively compared based on the health classification of the animals which were sampled. Among the adults, all of the biovariants were isolated most often from apparently healthy animals, with the exception of *M. haemolytica* $16^{\alpha E}$ (n = 1 isolate, from an animal with respiratory disease). This is consistent with domestic animal models of pasteurellosis, where P/M are a part of the normal flora and are associated with disease when favored by adverse combinations of host, agent, and environmental characteristics (Yates, 1982). In contrast, all biovariants from juveniles were isolated most often from animals classified as diseased, with the exception of *M. haemolytica* $10^{\alpha B}$ (n = 3 isolates). With the exception of three biovariants (*M. haemolytica* 9^{B}, *P.* (*B.*) *trehalosi* 2^{BG} , and *P.* (*B.*) *trehalosi* ^{2CDS}) which accounted for a total of 4 isolates, each of the biovariants isolated from clinically diseased juveniles was also isolated from apparently healthy adults. Whether this reflects the high percentage of submissions from juveniles classified as diseased or a greater susceptibility of juveniles to disease from these biovariants requires further research.

The odds of an isolate from an animal with respiratory disease being beta-hemolytic was greater (2.73, 95% CI 1.78 – 4.14) than the odds of an isolate from bighorn sheep without apparent disease being beta-hemolytic. This relationship was apparent for both adults (2.59, 95% CI 1.10 –6.07) and juveniles (2.85, 95% CI 1.83 –4.46). Therefore, this data set suggests that beta-hemolysis may have a prognostic value for P/M in bighorn sheep, much as it does for *Streptococcus* spp. (Nizet, 2002).

Data on the biovariants present in the general population of apparently healthy bighorn sheep was not available for this study. Consequently, it is not possible to determine whether the biovariants most commonly identified in diseased animals represent particularly pathogenic strains or are a reflection of the most common biovariants present in the general population of bighorn sheep. If the latter scenario is true, the diversity of isolates associated with sheep classified as diseased is consistent with models of pasteurellosis, where many P/M are a part of normal ruminant microflora (Yates, 1982; Confer *et al.*, 1988) and cause disease sporadically as opportunistic infections. The latter scenario would also be consistent with P/M as incidental isolates from diseased animals. Further work is needed to clarify whether one or a few isolates are responsible for causing respiratory disease in bighorn sheep.

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Table 3.1. Bacterial isolates from bighorn sheep oropharyngeal or nasal swabs submitted to the Caine Veterinary Teaching Center (1989-2004) by biovariant taxonomic status, bighorn sheep health status, and age class.

Bacterial isolates			Adult		Adult Total	Lamb		Lamb Total	Grand Total
Species	Type	Exceptions	Diseased	Healthy		Diseased	Healthy		
Haemophilus somnus						2		2	2
Mannheimia haemolytica	1	n/a[1]		14	14	4		4	18
		α		11	11				11
		αB		8	8				8
		aαBG		1	1				1
		αE		2	2				2
		E		3	3				3
	10	n/a[1]		5	5				5
		α	1	8	9	1		1	10
		αB		3	3	1	2	3	6
		αBE		1	1				1
		αBS		1	1				1
		αC		1	1				1
		αE		2	2				2
		αβ		1	1				1
		B		3	3				3
		BES		1	1				1
		βB		1	1				1
		E		3	3				3
	11	n/a[1]		5	5				5
		α		2	2				2
		αβG		1	1				1
		αGX		1	1				1
		αβ		1	1				1
	16	α		1	1				1
		αB		4	4				4
		αE	1		1				1
		B		1	1				1
	2	S		14	14				14
	3	n/a[1]		8	8	10	1	11	19
		α		7	7				7
		αB		3	3				3
		αBE		3	3				3
		αBEX		1	1				1
		αC		2	2				2
		αCD		1	1				1
		αE		2	2				2
		αES		1	1				1
		αG		1	1				1
		B		1	1				1
		BCX		1	1				1

Bacterial isolates			Adult		Adult Total	Lamb		Lamb Total	Grand Total
Species	Type	Exceptions	Diseased	Healthy		Diseased	Healthy		
Mannheimia haemolytica		BE	1	1	2				2
		BEX		2	2				2
		BX		1	1				1
		CDE		3	3				3
		E		3	3				3
	5	n/a[1]		18	18				18
		αB		1	1				1
		β		1	1				1
	6	n/a[1]		2	2				2
		α		1	1				1
		R		4	4				4
		RX		1	1				1
	7	n/a[1]		1	1	4		4	5
		B		1	1				1
		BX		1	1				1
	8	n/a[1]		5	5				5
		β		2	2				2
	9	αβB		3	3	1		1	4
		αBR		4	4				4
		αBRX		1	1				1
		αβ		2	2	4		4	6
		αβR		9	9				9
		αR		1	1				1
		B				1		1	1
		βR		1	1				1
	U	n/a[1]		1	1				1
		α		2	2				2
		αB		2	2				2
		αβBC		2	2				2
		αβBERX		1	1				1
		αβE		1	1				1
		αER		2	2				2
		αβ		7	7				7
		αR		1	1				1
		βBX		2	2				2
		βBEX		3	3	1		1	4
		βB		1	1				1
		αβB		2	2				2
		β	1	2	3	2		2	5
Pasteurella multocida	A			2	2	1		1	3
	B			6	6	1		1	7
	galli[2]			10	10				10
	septi[3]			2	2				2
	testu[4]			1	1				1
	U11			1	1				1

Bacterial isolates			Adult		Adult	Lamb		Lamb	Grand
Species	Type	Exceptions	Diseased	Healthy	Total	Diseased	Healthy	Total	Total
Pasteurella multocida	U16			1	1				1
	U2			3	3				3
	U23			1	1				1
	U6			5	5	2		2	7
	U8			3	3				3
Pasteurella (Bibersteinia) trehalosi	2	n/a[1]	13	197	210	29	7	36	246
		B	3	130	133	11		11	144
		αB		1	1				1
		BE		1	1				1
		BG				1		1	1
		BS		13	13				13
		C		4	4				4
		CD		1	1				1
		CDS				2		2	2
		CS		1	1				1
		E		6	6				6
		EDG		1	1				1
		GS		1	1				1
	4	n/a[1]	1	5	6	4		4	10
		B	1	6	7				7
		βBS		2	2				2
		BS		3	3				3
		CDE		1	1				1
		CDS		4	4				4
		DGS		1	1				1
		DS		1	1				1
		S		1	1				1
Not identified				8	8				8
Grand Total			22	653	675	82	10	92	767

[1] n/a = bacterial isolates that could not be classified by Type or Exceptions

[2] *Pasteurella multocida* subspecies *gallicida*

[3] *Pasteurella multocida* subspecies *stomatis*

[4] *Pasteurella multocida* subspecies *testudinis*

Table 3.2: The most common bacteria, at different classification levels, isolated from bighorn sheep submitted to the Caine Veterinary Teaching Center (1989-2004), by number of isolates, percentage of total isolates, and percentage of isolates from diseased animals

Classification level	Isolate	No. isolates (%)	Pct. Diseased[1]
Species (P = 0.41)	*Pasteurella (B.) trehalosi*[2]	452 (59%)	14%
	Mannheimia haemolytica	270 (35%)	12%
	Pasteurella multocida	35 (4.6%)	11%

Type (P = 0.34)	*Pasteurella (B.) trehalosi 2*	422 (55%)	14%
	Mannheimia haemolytica 3	53 (6.9%)	21%
	Mannheimia haemolytica 1	44 (5.7%)	10%
	Mannheimia haemolytica 10	35 (4.6%)	9%
	Mannheimia haemolytica U	33 (4.3%)	11%
	Pasteurella (B.) trehalosi 4	30 (3.9%)	20%
	Mannheimia haemolytica 9	26 (3.3%)	23%

Exception (biovariant)	*Pasteurella (B.) trehalosi 2**	246 (32%)	17%
	Pasteurella (B.) trehalosi 2[b]	144 (19%)	10%
	Mannheimia haemolytica 3*	19 (2.5%)	53%
	Mannheimia haemolytica 5*	18 (2.3%)	0%
	Mannheimia haemolytica 1*	18 (2.3%)	22%

[1]Percentage associated with diseased animals

[2] *Pasteurella* (*Bibersteinia*) *trehalosi*

* *Pasteurella* (*Bibersteinia*) *trehalosi* 2, *Mannheimia haemolytica* 3, *Mannheimia haemolytica* 5, and *Mannheimia haemolytica* 1 did not have any exceptions at the biovariant level

Table 3.3: Bighorn sheep bacterial isolates submitted to the Caine Veterinary Teaching Hospital (1989-2004), by year, age class, and health status.

	Adult		Lamb		Grand Total
Year	Diseased[1]	Healthy[2]	Diseased[1]	Healthy[2]	
1989	15	18		1	34
1990		49		9	58
1991		114	16		130
1992		24	2		26
1993		23			23
1994			28		28
1997		107			107
1998			20		20
1999		15	4		19
2000	3	148			151
2001		50	5		55
2002		96			96
2003	4		7		11
2004		9			9
Grand Total	22	653	82	10	767

[1]Bighorn sheep classified as diseased at the time of sample submission

[2] Bighorn sheep classified as healthy at the time of sample submission

Table 3.4. Yearly percentage (of the total number of isolates) of Pasteurellaceae biovariants with > 10 total isolates, and Grand Totals, from bighorn sheep submissions to the Caine Veterinary Teaching Center (1989-2004).

Biovariant	1989	1990	1991	1992	1993	1994	1997	1998	1999	2000	2001	2002	2003	2004	Grand Total[1]
PTRE 2 nβ*	15	12	47	12	30	7	12		5	7	20	42	9		157
PTRE 2^{B}	3	40		12	43		25	40	16	20	5	16	45	78	123
PTRE 2 β†	68	5	5	19		64			16	8	29				77
MHEM 3	3	7	4			29									15
MHEM 2^{S}			0.1	4						7	2	1			14
MHEM 1	3		0.1				7	20		1		1			12
PTRE 2^{BS}					4					7					12
MHEM 5		5									7	4			11
Other	8	31	44	53	23		56	60	63	50	63	36	46	22	290
Grand Total[1]	34	58	130	26	23	14	75	10	19	151	55	96	11	9	711

PTRE = *Pasteurella* (*Bibersteinia*) *trehalosi*

MHEM = *Mannheimia haemolytica*

[1] = numerical value

* = non-hemolytic

† = Beta-hemolytic

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Table 3.5. Biovariants that were more commonly associated with diseased bighorn sheep lambs.

	Adult		Lamb	
Biovariants	Diseased	Healthy	Diseased	Healthy
M. haemolytica 3†		8	10	1
M. haemolytica 7†		1	4	
M. haemolytica 9^{al}†		2	4	
M. haemolytica 9^{b}†			1	
P. (B.) trehalosi 2^{bg} *			1	
P. (B.) trehalosi 2^{cds} *			2	
P. (B.) trehalosi 2 *	13	197	29	7
P. (B.) trehalosi 2^{b} *	3	130	11	0

†*M. = Mannheimia*

**B. = Bibersteinia*

CHAPTER 4

DOMESTIC SHEEP PASTEURELLACEAE ISOLATES FROM DIAGNOSTIC SUBMISSIONS TO THE CAINE VETERINARY TEACHING CENTER (1990-2004)

Abstract

This study was conducted to identify Pasteurellaceae that were isolated from domestic sheep (*Ovis aries*) with respiratory disease, based on diagnostic samples submitted to a reference laboratory (Caine Veterinary Teaching Center) from 1990 – 2004. Submissions generally consisted of nasal or oropharyngeal samples from multiple animals, but submission information generally precluded associating samples or bacterial isolates with specific animals. Zero to multiple bacterial isolates were obtained from samples. Isolates (n = 878) were composed primarily of three Pasteurellaceae species: *Mannheimia haemolytica, Pasteurella multocida,* and *Pasteurella* (*Bibersteinia*) *trehalosi*. Among these three species, 117 biovariants were identified. Biovariants were identified 1 – 180 times. *Mannheimia haemolytica* 1 (20.5%) and *Pasteurella* (*Bibersteinia*) *trehalosi* 2 (15.7%) were the only biovariants sufficiently numerous to account for > 6 % of the total isolates. Most isolates were from sheep with signs of respiratory disease (n = 734), and most (76%) (n = 93) biovariants were identified most often in animals with signs of respiratory disease. However, some (28%) biovariants were isolated from both health categories (respiratory disease or apparently healthy) of sheep. Analysis of data at the species ($P = 0.04$) and type ($P < 0.0001$) taxonomic levels identified significant differences among isolates with respect to animal health categories. This suggested that *Pasteurella multocida* (4.6% of isolates) was the most likely to be associated with animals with respiratory disease when data was analyzed at the species level, whereas analysis at the type level suggested that *Mannheimia haemolytica* isolates were most likely to be associated with respiratory disease. This suggests that higher taxonomic levels of isolate classification may not be consistent with finer scale biovariant

classifications. There was not an association between isolate beta-hemolysis and animals with respiratory disease (P = 0.50; OR 0.88, 95% CI 0.60 – 1.29). While the inference of this study is limited, it provides a baseline list of biovariants that are associated with disease in domestic sheep.

Key words: retrospective, *Bibersteinia, Pasteurella*, *Mannheimia*, domestic sheep, disease, pasteurellosis

Introduction

Pasteurellosis is responsible for morbidity and mortality in nondomestic animals (Miller, 2001) that can result in substantial economic losses to livestock industries (Confer, 1993). Pasteurellaceae species commonly associated with respiratory disease epidemics are *Mannheimia* (*Pasteuerella*) *haemolytica* (Angen *et al.*, 1999), *Pasteurella* (*Bibersteinia*) *trehalosi* (formerly *P. haemolytica* biotype T)(Sneath & Stevens, 1990; Blackall *et al.*, 2007), or *Pasteurella multocida*. These species represent a heterogenous mix of strains that can be responsible for a range of clinical signs. The range in clinical signs may be the consequence of *Pasteurella* spp. and *Mannheimia* spp. (P/M) interacting with other pathogens, environmental factors, and host factors, as well as P/M characteristics (Czuprynski *et al.*, 2004; Zecchinon *et al.*, 2005; Dabo *et al.*, 2007). Of most concern are epidemics of pneumonic or septicemic pasteurellosis where P/M may act as a primary pathogen (Weekley *et al.*, 1998; Karunakaran *et al.*, 2008; Mishra *et al.*, 2000; Watson & Davies, 2002)

Several methods of classifying P/M exist. *Pasteuerella multocida* is conventionally classified by five capsular serogroups and 16 somatic serotypes (Confer, 1993). Subspecies and biotypes have also been identified biochemically (Biberstein *et al.*, 1991). *Mannheimia haemolytica* and *P. trehalosi* have also conventionally been classified by capsular antigens into serotypes (Confer, 1993; Blackall *et al.*, 2007). However, cross-agglutination or non-reactions with typing sera prevent classification of some isolates. Consequently, a biovariant classification scheme based on microbiological characteristics and biochemical utilization tests of isolates in culture was established to minimize the number of isolates which cannot be assigned to serotypes (Bisgaard & Mutters, 1986; Jaworski *et al.*, 1998). The biovariant classification scheme is hierarchical by species, type, and exceptions, with species being the broadest category, type being a subcategory of species, and exceptions being the most specific category (biovariant). The biovariant level of classification distinguishes among a greater number of P/M strains than do serotype classification schemes.

Limited data are available that associate specific P/M biovariants of domestic sheep with respiratory disease (Jaworski *et al.*, 1998). Consequently, the aim of this chapter is to identify P/M biovariants associated with domestic sheep classified as having respiratory disease. This chapter is a study of the P/M biovariants isolated from domestic sheep clinical samples submitted to a reference laboratory from 1990-2004. This is of relevance to pasteurellosis in domestic sheep. It is also germane to concerns that domestic sheep serve as asymptomatic reservoirs for P/M that cause disease in bighorn sheep (Council for Agricultural Science and Technology (CAST), 2008).

Methods

All bacterial samples from domestic sheep submitted to the Caine Veterinary Teaching Center (CVTC) from January 1, 1990 – December 31, 2004 were included in this study. Oropharyngeal and nasal swab samples that were placed in varying brands of commercial transport media for bacterial culture were submitted to CVTC by producers and veterinarians. Submissions generally consisted of multiple samples from multiple animals. Because submission information generally prevented associating samples with a specific animal or anatomical location, bacteriology results are reported only on an isolate basis. Each sample yielded zero to multiple bacterial isolates. Isolates described in this report were those which included more complete submission information, e. g., the date of submission, geographic location, and health classification (without clinical abnormalities or with signs of respiratory disease, hereafter referred to as healthy or diseased, respectively). Results were from a minimum of 104 different animals, based on the number of submissions; it is not possible to determine the total number of animals that were actually sampled.

Bacterial culture procedures

Samples were shipped overnight on cold packs and plated within 72 hours of collection.At CVTC the samples were inoculated onto nonselective Columbia blood agar (CBA), (Becton Dickinson & Co., Sparks, Maryland 21152, USA) containing 5% sheep blood, and selective Columbia blood agar with selective antibiotics, containing 5% bovine blood (Jaworski, et al., 1993) and incubated for 18 to 24 hr at 37°C in a 10% CO_2

atmosphere. Following incubation, representatives of each colony type were propagated on fresh CBA for species and biovariant classification.

Species and biovariant classification of bacterial isolates

Isolates were determined to be *M. haemolytica* or *P.* (*B*). *trehalosi*, as opposed to *P. multocida*, based on the following characteristics: urea- and indole-negative; oxidase-, nitrate-, glucose-, sucrose, and mannitol-positive; and failure to grow or poor growth on MacConkey's agar. *Mannheimia haemolytica* and *P.* (*B*). *trehalosi* were further distinguished if they were trehalose-negative-or trehalose-positive, respectively. Biovariant classification was done using a modification of a biochemical testing system developed for isolates from domestic animals (Bisgaard and Mutters, 1986), adapted for identifying isolates from wildlife (Jaworskiet al., 1998). Briefly, the wildlife method uses the results from 23 microbiological characteristics and biochemical utilization tests to separate isolates into biovariants which are hierarchically classified based on species, type, and exceptions. In addition, isolates with zones of hemolysis on blood agar were classified as beta-hemolytic. Following speciation and biovariant classification, isolates were stored frozen at -70 C in phosphate-buffered saline: glycerol (4:6 v/v, pH 7.2).

Statistics

Data from submission sheets were entered into a Microsoft Access database (Microsoft Corporation, One Microsoft Way, Redmond WA 98052 USA) at the CVTC and subsequently imported into an Access database developed for this study. For this study, accuracy was confirmed and corrections made by examining original laboratory

logs and submissions. Descriptive data and tables were developed directly from the database or after export into Microsoft Excel spreadsheet files.

Statistical analysis was conducted using data exported from the study database into SAS 9.2 (SAS Institute, Inc., Cary, NC 27513 USA). Exploratory analyses were conducted using the FREQ Procedure: chi-square analyses were considered to be significant at $P < 0.05$, and odds ratios were calculated for 2 X 2 tables. Fisher's exact test (n = 100,000 simulations) was used in place of chi-square analyses where there were multiple cells with expected values less than 5. Chi-square and Fisher's exact tests assume independence for data. Separate analyses were conducted for biovariants at each taxonomic level (species, type, and biovariant) to determine whether there was an association with the host animal's apparent health status. Chi-square analysis of biovariants was conducted to determine whether there was an association between the host animal's health classification and whether the isolate was beta-hemolyitic.

Results

Isolates (n = 878) were composed of *Actinobacillus* spp. (n = 3), Campylobacter spp. (n = 1), coliforms (n = 1), three species of Pasteurellaceae (*Mannheimia haemolytica* (n = 630), *Pasteurella multocida* (n = 41), and *Pasteurella* (*Bibersteinia*) *trehalosi* (n = 191)), and 11 isolates that could not be identified to species (Table 4.1). Among the three Pasteurellaceae species, 117 biovariants were identified (Table 4.1). The maximum number of times a single biovariant was isolated was 180 (20.5% of isolates) for *M. haemolytica*1 (Table 4.2). Ninety-three biovariants were associated with animals classified as diseased.

The majority of isolates (58.2%) were from Idaho (Table 4.3). As Idaho was the only state with >5 isolates for both animal health classifications, statistical analyses were not conducted on the basis of state. The number of isolates per year varied from 1-432 (Table 4.4), and the biovariants isolated varied temporally (Table 4.5). As only one year (1994) had >5 values for submissions from both health classifications, statistical analyses based on year were not attempted.

Evaluation of data at the species taxonomic level identified *M. haemolytica* (72%), *P.* (*B.*) *trehalosi* (22%), and *P. multocida* (5%) as the most common species, with *P. multocida* having the highest percentage (98%) of samples from sheep classified as diseased (Table 4.2). There was a significant difference (P = 0.04) among the bacterial species by disease status. Sixteen isolates (2% of isolates) that were not identified to species or which had <5 isolates for an animal health classification were not included in the analysis.

Evaluation of data at the type taxonomic level identified *M. haemolytica* 1 (27%), *P.* (*B.*) *trehalosi* 2 (18%), and *M. haemolytica* U (9%) as the most common species, with *M. haemolytica*1 having the highest percentage (94%) of samples from sheep classified as diseased (Table 4.2). There was a significant difference (P <0.01), using the Fisher's exact test, among these isolates by host animal disease classification. Fisher's exact simulations were based on a sample size of 862 isolates.

Evaluation of data at the biovariant (exception) level identified *M. haemolytica* 1 (21%), *P.* (*B.*) *trehalosi* 2 (16%), and *M. haemolytica* 11 (6%) as the most common isolates, with *M. haemolytica* 1 having the highest percentage (96%) of samples from sheep classified as diseased (Table 4.2). No analysis was conducted for biovariants based

on health classification because only four biovariants (*M. haemolytica* 16^{AE}, *M. haemolytica* 16^{E}, *M. haemolytica* 1^{G}, *M. haemolytica* U^{β}) , consisting of 57% of the data, had >5 values in both animal health classification cells.

The odds of an isolate from an animal with respiratory disease being beta hemolytic were estimated to be similar (odds ratio 0.878, 95% CI 0.599 – 1.287, P = 0.5049) to the odds of an isolate from animals without apparent disease being beta hemolytic.

Discussion

This data set is a comprehensive list of Pasteurellaceae biovariants isolated from domestic sheep diagnostic samples submitted to the CVTC (Table 4.1). Only 28% of biovariants were associated with both health classifications of sheep (clinically diseased and apparently healthy), and isolates could not be associated with individuals. This was a retrospective study of clinical submissions where swab collection methods, swab type, animal health classification, and transport media were not standardized. In addition, most samples were from Idaho (Table 4.3), submitted in 1994 (Table 4.4), and there was substantial yearly variation in the biovariants present (Table 4.5). Consequently, it is unlikely that the assumptions of random samples, independent observations, and similar distributions of data in comparisons were met for statistical analyses. Therefore, although laboratory protocols were consistent and it is assumed that bacterial classifications are stable, caution is warranted on the degree of inference possible from these results. However, this data is of value for a preliminary assessment of Pasteurellaceae strains associated with disease in domestic sheep.

Ninety three biovariants were associated with sheep classified as diseased. *Pasteurella multocida* and *M. haemolytica* have previously been associated with respiratory disease in domestic sheep (Watson & Davies, 2002; Odugbo *et al.*, 2004), while there is little previous documentation of an association between *P.* (*B.*) *trehalosi* and disease in domestic sheep. These taxonomic categories of bacterial isolates are often used in diagnoses for respiratory disease. However, they actually represent an assemblage of bacterial lineages that may not have similar levels of pathogencity.

Although it is presumed that narrower taxonomic or molecular classification schemes may be more useful for disease investigations, this has not been established for Pasteurellaceae in domestic sheep. As a preliminary means of addressing this, isolates were evaluated at each taxonomic level (species, type, exceptions)(Table 4.2). *Mannheimia haemolytica* was the most numerous isolate at each taxonomic level, followed by *P.* (*B.*) *trehalosi*. However, the association of Pasteurellaceae with respiratory disease is of greater interest than the number of times a type of Pasteurellaceae is identified. While the association between isolates and animals classified as diseased are significantly different at the species ($P = 0.0434$) and type taxonomic levels ($P < 0.0001$), the bacteria (*P. multocida* and *Mannheimia haemolytica* 1, respectively) responsible for these results differ. At the biovariant level, *Mannheimia haemolytica* 1 (96%) and *Mannheimia haemolytica* 11 (94%) appear to have the greater percentage of isolates associated with animals classified as diseased (Table 4.2). It is also notable that there is a large gap between the most common biovariants in this study, and the biovariants that were less commonly identified; *Mannheimia haemolytica* 1 and *P.* (*B.*) *trehalosi* 2 account for 36% of the isolates, and none of the other 115 biovariants

accounted for > 7% of the total (Table 4.2). These discrepancies suggest that higher taxonomic levels of classification may aggregate Pasteurellaceae isolates such that it is difficult to accurately identify Pasteurellaceae lineages that are most commonly associated with disease.

The preponderance of samples from animals with respiratory disease is likely responsible for the low percentage (28%) of biovariants identified in animals with both health classifications. This precludes quantitative estimates of Pasteurellaceae that are most associated with respiratory disease. Additional information that is needed for such estimates is baseline data on the population from which the samples are collected (the denominator). As the most pathogenic Pasteurellaceae may not have the greatest population level effects, it is also important to obtain epidemiological data that can place quantitative estimates of pathogenicity in perspective. A lack of correspondence between Pasteurellaceae pathogencity and population level effects could occur due to differing levels of transmission, risks of exposure, or other factors.

Beta-hemolysis is sometimes used as an index of isolate pathogenicity in clinical settings and was evaluated as a potential indicator of the pathogenic potential of isolates. There was a lack of association between isolate beta-hemolysis and animals with respiratory disease (OR 0.878, 95% CI 0.599 – 1.287). Consequently, use of beta-hemolysis as an index of bacterial pathogenicity in domestic sheep may be more appropriate for *Streptococcus* spp. than for Pasteurellaceae (Nizet, 2002).

Data on the biovariants present in the general population of apparently healthy domestic sheep was not available for this study. Consequently, it is not possible to determine whether the biovariants most commonly identified in diseased animals

represent particularly pathogenic strains or are a reflection of the most common biovariants present in the general population of domestic sheep. If the latter scenario is true, the diversity of isolates associated with sheep classified as diseased is consistent with models of pasteurellosis, where many Pasteurellaceae are a part of normal ruminant microflora (Yates, 1982; Confer *et al.*, 1988) and cause disease sporadically as opportunistic infections. The latter scenario would also be consistent with Pasteurellaceae as incidental isolates from diseased animals. Further work is needed to clarify whether one or a few isolates are responsible for causing respiratory disease in domestic sheep.

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Table 4.1. Bacterial isolates from domestic sheep oropharyngeal or nasal swabs submitted to the Caine Veterinary Teaching Center (1990-2004) by biovariant taxonomic status and domestic sheep health status.

Species	Type[1]	Exceptions[1]	Diseased	Healthy	Grand Total
Actinobacillus spp.	n/a	n/a	2	1	3
Campylobacter spp.	n/a	n/a	1		1
Coliform	n/a	n/a	1		1
Mannheimia haemolytica	1	α	9	1	10
		αB	4		4
		αG	1		1
		B	3		3
		E	1		1
		EG	2		2
		G	29	5	34
		n/a	172	8	180
	10	α	9	1	10
		αG		1	1
		C		1	1
		n/a	4	1	5
	11	α	2	1	3
		αBE	1		1
		αE		1	1
		αβ		1	1
		B		2	2
		BE	1		1
		E	10	1	11
		n/a	50	3	53
	16	α	3	1	4
		αB	3		3
		αBE	12		12
		αβBE	1		1
		αE	5	7	12
		αEG	10		10
		αG	1		1
		BE	2		2
		E	11	2	13
		EG	9		9
		G	2		2
		βBE		1	1
		n/a	3		3
	2	S	1		1

Species	Type[1]	Exceptions[1]	Diseased	Healthy	Grand Total
Mannheimia haemolytica	3	ABCDE	1		1
		B		1	1
		n/a	18	7	25
	5	α	1	2	3
		αB		1	1
		αBC		1	1
		αBCD		1	1
		αβ		2	2
		B		2	2
		BCD	1		1
		BD		1	1
		CDS		1	1
		E	1		1
		βB		1	1
		n/a	15	3	18
	6	α	1		1
		αB	3	2	5
		αR	2		2
		R	1		1
		RX	1		1
	7	B	5	2	7
		BG	2		2
		βBX	5		5
		BX	16	2	18
		G	1		1
		X	27	4	31
		n/a	6	3	9
	8	B	6	2	8
		βB	1		1
		n/a	2	7	9
	9	αβ	1	1	2
		B	1		1
		β		1	1
	u	αBE	1		1
		αBER	4		4
		αβBG	1		1
		αβB	13		13
		αβBX	3		3
		αER	1		1
		αβ	1	7	8
		αβB		2	2
		αβX	1		1
		βBE	6		6

Species	Type[1]	Exceptions[1]	Diseased	Healthy	Grand Total
Mannheimia haemolytica	u	βB	5		5
		βBX	8	1	9
		E	1		1
		βE	1		1
		β	6	9	15
		βBE		1	1
		βBEX		1	1
		βBX		1	1
		βE		2	2
		βX	1		1
Pasteurella multocida	a	n/a	10		10
	b	n/a	6		6
	canis[2]	n/a	4		4
	gallicida[3]	n/a	1		1
	stomatis[4]	n/a	1		1
	testudinis[5]	n/a	1		1
	U12	n/a	1		1
	U16	n/a		1	1
	U18	n/a	4		4
	U20	n/a	3		3
	U26	n/a	1		1
	U6	n/a	7		7
	n/a	n/a	1		1
Pasteurella (Bibersteinia) trehalosi	2	αB	1		1
		B	1		1
		C	2		2
		CD	6		6
		CDES	1		1
		CDS	4		4
		D	1		1
		E	5	1	6
		S	1		1
		n/a	115	23	138
	4	BCDS	3		3
		CD	1		1
		CDES	2	1	3
		CDS	3	2	5
		CS		1	1
		S		1	1
		n/a	1	4	5
Not Identified	n/a	n/a	11		11
Grand Total			734	144	878

[1]n/a = bacterial isolates that could not be classified by Type or Exceptions

[2] *Pasteurella multocida* subspecies *canis*

[3] *Pasteurella multocida* subspecies *gallicida*

[4] *Pasteurella multocida* subspecies *stomatis*

[5] *Pasteurella multocida* subspecies *testudinis*

Table 4.2: The most common biovariants, at different classification levels, isolated from domestic sheep samples submitted to the Caine Veterinary Teaching Hospital (1990 - 2004), by number of isolates, proportion of total isolates, and proportion of isolates associated with disease.

Classification level	Isolate	No. isolates (%)	Pct. Diseased[1]
Species	*Mannheimia haemolytica*	630 (71.8%)	83%
(P = 0.04)	*Pasteurella (B.) trehalosi*[2]	191 (21.7%)	82%
	Pasteurella multocida	41 (4.7%)	98%

Type	*Mannheimia haemolytica* 1	235 (26.8%)	94%
(P <0.01)	*Pasteurella (B.) trehalosi* 2	161 (18.3%)	69%
	Mannheimia haemolytica U	77 (8.8%)	69%

Exception	*Mannheimia haemolytica* 1*	180 (20.5%)	96%
(biovariant)	*Pasteurella (B.) trehalosi* 2*	138 (15.7%)	83%
	Mannheimia haemolytica 11*	53 (6%)	94%
	Mannheimia haemolytica 1^{g}	34 (3.9%)	85%
	Mannheimia haemolytica 7^{x}	31 (3.5%)	87%
	Mannheimia haemolytica 7^{bx}	18 (2.1%)	89%

[1]Percentage associated with diseased animals

[2] *Pasteurella* (*Bibersteinia*) *trehalosi*

* *Mannheimia haemolytica* 1, *Pasteurella* (*Bibersteinia*) *trehalosi* 2, and *Mannheimia haemolytica* 11 did not have any exceptions at the biovariant level

Table 4.3: Domestic sheep bacterial isolates from samples submitted to the Caine Veterinary Teaching Center (1990-2004), by state and animal health classification.

	Colorado		Idaho		Montana		Nevada		Oregon		Washington		Wyoming		
Bacterial Species	D^1	H^2	D^1	H^2	D^1	H^2	D^1	H^2	D^1	H^2	D^1	H^2	D^1	H^2	Grand Total
MHEM	1	2	285	68	-	38	141	-	19	-	34	-	42	-	630
PMULT	-	-	17	1	-	-	18	-	-	-	5	-		-	41
PTRE	-	-	109	18	-	16	31	-	7	-	2	-	8	-	191
Not identified	-	-	12	1	-	-	2	-	-	-	1	-		-	16
Grand Total	1	2	423	88	0	54	192	0	26	0	42	0	50	0	878

D^1 = Isolates collected from animals with apparent respiratory disease

H^2 = Isolates collected from apparently healthy animals

MHEM = *Mannheimia haemolytica*

PMULT = *Pasteurella multocida*

PTRE = *Pasteurella (Bibersteinia) trehalosi*

Table 4.4: Domestic sheep bacterial isolates by year at the Caine Veterinary Teaching Center (1990-2004).

Year	Diseased[1]	Healthy[2]	Grand Total
1990	33		33
1991	31		31
1992	81		81
1993	57		57
1994	412	20	432
1995	79		79
1996	16		16
1997	8		8
1998	6		6
1999	2	2	4
2001	1		1
2002	4		4
2003		67	67
2004	4	55	59
Grand Total	734	144	878

[1]Domestic sheep classified as diseased at the time of sample submission

[2] Domestic sheep classified as diseased at the time of sample submission

Table 4.5. Yearly percentage (of the total number of isolates) of Pasteurellaceae biovariants with > 10 total isolates, and Grand Totals, from domestic sheep submissions to the Caine Veterinary Teaching Center (1990-2004).

Biovariant	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2001	2002	2003	2004	Grand Total[1]
MHEM 1	15	42	41	5	20	24	44	75	67	25				2	178
PTRE 2†		10		14	15	8	6						16	8	97
MHEM 11			2	28	7	1	6		17	25				2	53
PTRE 2*			7	4	6	4							6	2	41
MHEM 1^{G}	45		2		0	1	19			25			6		28
MHEM 3				14	2								7	3	25
MHEM 7^{X}					4	6									22
MHEM 5			5	4	2	1								5	18
MHEM 7^{BX}					3	6									16
MHEM U^{L}			1		1								12	2	15
MHEM U^{ABL}					3										13
MHEM 16^{AE}					1	3							7		11
MHEM 16^{E}				2	2	1									11
PTRE 11^{E}					2								1		11
MHEM 16^{AEG}	21	3			0										10
PMULT A	6		1		1	1									10
Other	12	45	40	30	30	43	25	25	17	25	100	100	43	76	319
Grand Total[1]	33	31	81	57	432	79	16	8	6	4	1	4	67	59	878

MHEM = *Mannheimia haemolytica*
PMULT = *Pasteurella multocida*
PTRE = *Pasteurella* (*Bibersteinia*) *trehalosi*
[1] = numerical value
* = non-hemolytic
† = Beta-hemolytic

CHAPTER 5

DESCRIPTION OF DOMESTIC LIVESTOCK OPERATIONS AND VIEWPOINTS

Introduction

A questionnaire was administered to domestic sheep and goat operators with the aim of characterizing the populations studied in Chapter 6. This was developed as a pilot project, in anticipation that field work associated with this dissertation might identify respiratory disease risk factors that could be managed. It also provides information on the domestic animal populations studied for this dissertation. This questionnaire was developed and administered in consultation with the United Stated Department of Agriculture, Veterinary Services. This questionnaire addressed domestic livestock management practices, interactions with other populations and species, and producer attitudes.

Methods

Domestic livestock population characteristics were compiled from questionnaires based on previous NAHMS (USDA, National Animal Health Monitoring System) questionnaires (USDA, 2001a; USDA, 2001b; USDA, 2002b; USDA, 2003), and were verbally administered after biological samples were collected (Appendix 1). The questionnaire addressed the operation's size, disease occurrence, causes of animal loss, disease control efforts, and potential routes of agent transmission, as well as exploratory questions regarding producer perceptions about bighorn sheep and management conflicts. Means and ranges are reported for questionnaire and census data.

Results

Six domestic sheep populations (152 individuals sampled) were located at the wildlife-livestock interface and six populations (219 individuals sampled) were not. The

single goat population of Spanish meat goats (n = 45 individuals sampled) sampled was at the wildlife-livestock interface. Domestic sheep populations ranged in size from 25 – 4000 females, with one noninterface herd numbering 4000, and all other populations with <2000 females (Table 5.1). Multiple breeds of sheep were studied (Table 5.2). More interface populations (67%) reported that they had larger populations than five years previous than non-interface populations.

With the exception of one domestic sheep population (> 4000 animals) with summer grazing at 3048 m, populations were managed at 981 - 1707 m elevation (Table 5.1). All but one operation reported checking their animals at least once daily during winter, as well as provision of supplemental feed, and all but three operations checked animals at least once daily during summer. Most operations used private land during winter (83%) and summer (75%). Populations were managed on herded open range, fenced range, and in farm settings, with some variation by season.

Calculated indices of fecundity were similar for operations at the interface with bighorn sheep and > 14.5 km from the interface (Table 5.3). With the exception of two interface domestic sheep operations of ≤ 100 animals that reported breeding animals in August, and one non-interface operation that completed breeding in January, breeding start and end dates were similar for interface and noninterface domestic sheep populations (Table 5.1). Breeding season length of 29 or 30 days was reported by 75% of operations. Lambing season started in December for one interface domestic sheep population, with all other births occurring March – June. The range in the age at weaning was similar for interface and noninterface populations, although one interface population did not report this parameter. All operations managed mothers and offspring in pens for the first 24 h post-partum. Goats gave birth on open range and remained on open range.

Most (77%) operations permitted visitor access to sheep-raising areas (Table 5.1). Biosecurity measures for visitors included restricting access to some areas (n = 1), monitoring visitor activity (n = 1), foot covers if visitors had been at other operations (n = 1), and prevention of access if visitors had been on other operations (n = 1). Transfer of animals between populations consisted primarily of breeding males. All operations reported varying levels of contact with other domestic or wildlife species.

Less than half of all operations (46%) had received private practice or government veterinary consultation in the previous year for at least one reason. Diagnostic laboratories were not utilized. Additional resources for information included extension agents and nutritionists

Half of domestic sheep operations treated for ectoparasites, and 75% used at least one type of vaccine. (Table 5.1). Although only four operations tested for endoparasites, all but one goat operation treated for endoparasites. Management plans were developed in response to specific disease conditions at a higher proportion for interface operations (83%) than in noninterface operations (38%). Spring was the most common season to observe respiratory disease.

Every livestock operation experienced animal losses from at least one cause during the previous year. Multiple predator control strategies were employed. Effective strategies for segregating wildlife from livestock operations were limited.

A range of opinions existed regarding disease transmission between bighorn and domestic sheep, as well as knowledge of management options. Livestock operators indicated that they felt that the greatest sources of conflict between bighorn and domestic sheep was the 14.5 km buffer, due to decreased grazing range or management options for domestic sheep (n = 5), unscientific policies (n = 2), or politics (n = 1). Four operations

(three at the interface) felt that there was no conflict. All but one interface operation indicated that bighorn sheep are an important and valued part of the environment of Montana. All operators would be willing to use a treatment or management protocol to eliminate transmission of disease between bighorn and domestic sheep, but only one operation was willing to accept alternate grazing allotments. Options for decreasing conflict between bighorn and domestic sheep interests that were listed by producers included use of guard dogs, development of science-based management strategies, use of a *Pasteurella* vaccine, shooting bighorn sheep that leave their appropriated range, and changes in livestock housing.

Discussion

A range in domestic livestock population sizes, breeds, and management practices were documented in this study. Although less common breeds such as Romanov, Shetland, and Romney were included in the study, they composed < 8% of the animals sampled. As might be expected from Montana's low human population, this study's livestock populations tended to be large and kept on rangeland, with about one third of the study populations kept as farm flocks. This is in contrast to regional (45%) and national data (78 %) for farm flocks (USDA, 2003). Livestock were kept on primarily on private land, although contention over grazing allotments on public land has been the focus of debate at the bighorn/domestic sheep interface (United States Geologic Survey/Bureau of Reclamation Office, 2006). Half of the operations reported having more animals than 5 years previously, as compared to 24% of operations in the region reporting increased animal numbers (USDA, 2002b). The large size of operations, the location of operations on private land, and operations that are increasing in size suggests

the potential for domestic and bighorn sheep to intermingle where public land inhabited by bighorn sheep is adjacent to livestock operations.

It is possible that domestic sheep operations willing to cooperate with this study were more intensively managed and successful than other operations. Examples consistent with this hypothesis are lambing seasons that were generally short (30 d) and the separation of post-partum ewes with their lambs into pens. Such management practices may account for a relatively high number of weaned lambs per ewe (1.6) (USDA, 2001a; USDA, 2002a; USDA, 2003). Contrasting evidence might be lamb losses that are greater than national averages, although this may be more a reflection of closer monitoring and better records than of higher losses (USDA, 2003).

There are a number of disease conditions that can affect domestic sheep production. Use of veterinary or diagnostic laboratory expertise is not extensive for the populations in this study, due in part to limited domestic sheep veterinary expertise in the region and producer cost:benefit concerns (USDA, 2002b). This might be countered by producer's greater use of extension agent expertise. In comparison to regional data, high proportions of operations administered vaccinations and anthelmintics, and had treatment plans for addressing specific diseases.

Agent transmission at the bighorn/domestic sheep interface has garnered much attention. However, interpopulation transmission of agents with pathogenic potential is also of concern within the domestic sheep industry, livestock industries in general, and wildlife interests. In the populations studied, there was limited exchange of animals between populations. This limits opportunities for agent transmission, but is in contrast to limited biosecurity measures for human visitors and trailers. The limited exchange of sheep and goats between populations is also contrasted by potential opportunities for

agent transmission from wildlife and other domestic species across fence lines, on pasture, and in pens. The limited recognition of disease outbreaks due to interactions with these other species may reflect limited transmission of agents with pathogenic potential. It is also possible that the impact of these interactions may not be recognized, or may be limited to infrequent instances where novel agents are introduced into naïve populations.

In addition to losses due to disease, livestock operations also lose animals to multiple predator and non-predator causes (USDA, 2007; USDA, 2005). These losses can compromise livestock operation profitability and viability. Some predator control strategies (ie. shepherds, guard dogs, etc.) may have the potential to be developed as strategies for separating bighorn sheep and other wild hoofstock from livestock, but none have been demonstrated to date.

Producers expressed a range of opinions and knowledge regarding bighorn sheep and bighorn sheep management. This type of information can be used by programs intended to gain support for wildlife management objectives (Riley & Decker, 2000). However, responses from four operations that indicated that there were no conflicts between domestic and bighorn sheep interests suggests that either knowledge of bighorn sheep is limited, or the belief that such conflicts are insubstantial. The latter scenario illustrates a potential limitation for education programs. This is because a target population's interpretations of available data may differ from that of an educational program due to differing value systems.

Among the livestock operations studied, there was widespread support for bighorn sheep and they were perceived as a valuable species. There was also willingness among producers to consider domestic sheep treatments or management actions that would minimize disease transmission between bighorn and domestic sheep. This suggests

that there may be strategies that can minimize conflict between domestic and bighorn sheep interests. However, there is extensive use of private land, limited interest in alternative grazing allotments, and concerns about management policies that are not science-based. This suggests that management strategies for minimizing conflict at the bighorn/domestic sheep interface must be appropriate and well conceived.

Transmission of agents between different populations of animals, particularly closely related species such as bighorn and domestic sheep, is a general concern and is the basis for regulations on the international movement of animals (Zepeda *et al.*, 2001). The occurrence, frequency, and impact of such transmission at the bighorn/domestic sheep interface is uncertain (United States Geologic Survey/Bureau of Reclamation Office, 2006). This study provides background data on domestic sheep operations and indicates the potential for acceptance of management strategies that decrease conflict at the bighorn/domestic sheep interface.

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Table 5.1: Responses to a questionnaire (Appendix 1) administered to domestic livestock collaborators, with interface and non interface populations, concerning herd characteristics, herd management, reproductive management, biosecurity, treatment and prevention of disease, animal loss, and producer opinions.

Question		Non-interface populations (n = 6)	Interface populations (n = 6)	Goats (n = 1)
I. Herd Characteristics				
Number of ewes ≥ 1 y	Mean ± S.D.	1030 ± 1509.1	467.1 ± 656.7	925
	Range	30 – 4000	25 – 1780	
Number of rams ≥ 1 y	Mean ± S.D.	36 ± 57.1	12 ± 13.7	19
	Range	2 – 150	1 – 40	
Replacement lambs < 1 y	Mean ± S.D.	520 ± 976.5	156.3 ± 227.5	430
	Range	10 – 2500	3 – 540	
Market lambs < 1 y	Mean ± S.D.	745 ± 1149.1	332.4 ± 474.5	450
	Range	0 – 3000	0 – 1360	
Number of ewes bred	Mean ± S.D.	1039.5 ± 1506.5	393.3 ± 521	940
	Range	30 - 4000	28 – 1330	
Number of lambs born	Mean ± S.D.	1514.2 ± 2384.6	542.6 ± 726.4	980
	Range	70 - 6300	41 – 2025	
Number of lambs weaned	Mean ± S.D.	1351.1 ± 2117.8	495.1 ± 662.9	900
	Range	65 - 5600	39 - 1845	
No. bighorn-domestic sheep hybrids (last 5 years)		0	0	0
Number of animals vs. 5 years previous	More	2	4	0
	Same	0	1	0
	Less	4	1	1
II. Herd Management				
Winter elevation (m)	Mean ± S.D.	1278 ± 204	1,253 ± 242	1311
	Range	1006 - 1524	981 - 1707	0
Winter land type	Public	2	0	0
	Private	4	6	1
Winter supplemental feed	Provided/not	5/1	5/1	1/0
Winter management	Herded/open range		1	1
	Fenced range	4	3	0
	Farm	2	2	0
Winter monitoring frequency	≥ 1 time per day	5	6	1
	≥ 1time per week	1	0	0
Summer elevation (m)	Mean ± S.D.	1620 ± 743	1318 ± 255	1524
	Range	1006 - 3048	981 - 1707	0
Summer land type	Public	3	(10%)	1
	Private	3	6(90%nb)	0
Summer supplemental feed	Provided/not	2/4	1/5	1/0
Summer management	Herded/open range	2	2	1
	Fenced range	2	2	0
	Farm	2	2	0
Summer monitoring frequency	≥ 1 time per day	4	5	1
	≥ 1time per week	2	1	

Question		Non-interface populations (n = 6)	Interface populations (n = 6)	Goats (n = 1)
III. Reproductive Management				
Start of previous breeding season		October 6-November 10	August 1 – November 15	November 15
End of previous breeding season		November 15 – January 10	November 15 – December 15	December 15
Start of previous birthing season		March 1 – April 6	December 15 – April 15	April 15
End of previous birthing season		April 20 – June 1	April 1 – May 4	May 15
Length of breeding season (d)	Mean ± S.D.	40 ± 14.5	49 ± 32.8	30
	Range	30 – 61	30 - 121	
Average age at weaning	Mean ± S.D.	147 ± 22	158 ±35	180
	Range	120 - 180	100 - 180	
Animal location during birth	Open range	5	0	1
	Pasture	1	0	0
	Pens	0	6	0
Management of mother-offspring first 24 hours	Separate pen by themselves	4	6	(open range)
	Separate pen with other pairs	2	0	0
IV. Biosecurity				
Visitors allowed in birthing areas	Yes/no	5/1	5/1	0/1
Adult females/lambs added to population in previous year	Yes/no	1/5	1/5	1/0
Adult males added to population in previous year	Yes/no	4/2	3/3	0/1
Animals that left for shows, breeding, or exhibitions and returned to population	Yes/no	0/6	0/6	0/1
Graze with other domestic sheep populations	Yes/no	0/6	0/6	0/1
Breeding males temporarily on premises	Yes/no	2/4	1/5	0/1
Sheep from other populations on premises for shearing or breeding	Yes/no	0	1/5	0/1
Other contact with sheep from other populations	Yes/no	0	0	0
Transportation of animals in the previous year	Yes/no	3/3	1/5	0/1
Access to operation of bighorn sheep in previous year	Fenceline contact only	0	1	0
	On pasture at different times	1*domestic sheep summer range used by in winter	2	1
	On pasture at same time	0	1	1

Question		Non-interface populations (n = 6)	Interface populations (n = 6)	Goats (n = 1)
IV. Biosecurity (continued)				
	Contact in sheds, food or water pans, holding pens	0	1	1
Access to operation of Rocky Mountain goats in previous year	Fenceline contact only	0	0	0
	On pasture at different times	1*domestic sheep summer range used by in winter	0	0
	On pasture at same time	0	0	0
	Contact in sheds, food or water pans, holding pens	0	0	0
Access to operation of deer in previous year	Fenceline contact only	1	0	0
	On pasture at different times	2	1	1
	On pasture at same time	0	4	0
	Contact in sheds, food or water pans, holding pens	5	1	1
Access to operation of elk in previous year	Fenceline contact only	0	0	0
	On pasture at different times	1	2	0
	On pasture at same time	1	3	0
	Contact in sheds, food or water pans, holding pens	2	3	0
Access to operation of moose in previous year	Fenceline contact only	0	0	0
	On pasture at different times	0	1	1
	On pasture at same time	1	2	
	Contact in sheds, food or water pans, holding pens	0	1	1
Access to operation of pronghorn in previous year	Fenceline contact only	0	0	0
	On pasture at different times	2	2	1
	On pasture at same time	3	1	0
	Contact in sheds, food or water pans, holding pens	0	1	1

Question		Non-interface populations (n = 6)	Interface populations (n = 6)	Goats (n = 1)
IV. Biosecurity (continued)				
Access to operation of bison in previous year	Fenceline contact only	0	0	0
	On pasture at different times	0	0	0
	On pasture at same time	0	0	0
	Contact in sheds, food or water pans, holding pens	0	0	0
Access to operation of other nondomestic hoofstock in previous year	Fenceline contact only	0	0	0
	On pasture at different times	0	0	0
	On pasture at same time	0	0	0
	Contact in sheds, food or water pans, holding pens	0	0	0
Access to operation of horses in previous year	Fenceline contact only	0	1	0
	On pasture at different times	1	2	1
	On pasture at same time	4	2	1
	Contact in sheds, food or water pans, holding pens	1	2	1
Access to operation of domestic goats in previous year	Fenceline contact only	0	0	0
	On pasture at different times		1	0
	On pasture at same time	1	1	0
	Contact in sheds, food or water pans, holding pens	1	1	0
Access to operation of cattle in previous year	Fenceline contact only	0	1	0
	On pasture at different times	1	2	1
	On pasture at same time	4	2	1
	Contact in sheds, food or water pans, holding pens	2	2	1

Question		Non-interface populations (n = 6)	Interface populations (n = 6)	Goats (n = 1)
IV. Biosecurity (continued)				
Access to operation of llamas/alpacas in previous year	Fenceline contact only	0	0	0
	On pasture at different times	0	0	0
	On pasture at same time	2	2	1
	Contact in sheds, food or water pans, holding pens	1	3	1
Access to operation of poultry (chickens or turkeys) in previous year	Fenceline contact only	0	0	0
	On pasture at different times	0	0	0
	On pasture at same time	0	0	0
	Contact in sheds, food or water pans, holding pens	2	1	1
V. Veterinary Treatment or Prevention		0	0	0
Veterinary consultation in the previous year	Disease diagnosis	3	1	0
	Disease prevention	4	1	0
	Information on nutrition	3	1	1
	Production management	1	0	0
	Lambing abnormalities	4	1	0
	Lameness	1	1	0
	Private practitioner	4	1	1
	Government veterinarian	2	1	
Extension agent visit in the previous year		4	1	1
Nutritionist visit in the previous year		2		
Lamb vaccination for Clostridia	Yes	4	4	1
Other lamb vaccines		Campylobacter Contagious echtyma Vibrio Pasteuerellosis	Contagious echthyma Vibrio Leptospirosis	0
Fecal parasite testing	Yes	2	2	0
Anthelmintic administration	Yes	6	6	0

Question		Non-interface populations (n = 6)	Interface populations (n = 6)	Goats (n = 1)
V. Veterinary Treatment or Prevention (continued)				
Reason for anthelmintic administration	General prevention	5	5	0
	Worms seen	1	2	0
	Fecal testing	1		0
	Poor animal condition		3	0
Frequency of anthelmintic administration		1 - 3	1 - 4	0
Multiple types of anthelmintics administered		2	2	0
External parasite treatment	General prevention	3	3	0
	Ectoparasites seen	2	3	1
Seasons respiratory disease observed	Winter	1	1	0
	Spring	3	2	1
	Summer	1	1	1
	Fall	1	1	
Perceived causes of respiratory disease	Bacteria	3	1	0
	Dust ± high ammonia levels	0	2	1
VI. Animal Loss During the Previous Year				
Causes	Predators	4	4	1
	Respiratory disease	4	3	1
	Nutritional disease	1	3	1
	Gastrointestinal disease	1	3	1
	Other disease	1	0	0
	Bad weather	5	4	1
Strategies for guarding from predators	Shepherds	2	3	1
	SAC	2	4	0
	donkey	1	1	0
	dog	3	4	1
	M-44	2	2	1
	toxic collars	0	0	0
	shooting	3	4	1
	aerial gunning	3	4	1
	fencing	3	1	1
	sound devices	1	0	0
	trap/snare	4	1	1

Question		Non-interface populations (n = 6)	Interface populations (n = 6)	Goats (n = 1)
VII. Strategies for separating from wildlife				
Bighorn sheep	yes/effective	0	0	0
Bison	yes/effective	0	1/1	1/1
Pronghorn	yes/effective	1/1	0	0
Deer	yes/effective	1/1	1/0	0
Elk	yes/effective	1/1	1/0	0
VIII. Exploratory Opinion Questions				
Is the 14.5 km buffer effective for preventing disease transmission?	yes/no/don't know	1/3/2	1/2/3	1/0/0
Should bighorn and domestic sheep always be kept separate	yes/no/don't know	2/3/1	2/2/2	0/1/0
Bighorn sheep are important and valuable for Montana	yes/no/don't know	6/0/0	5/0/1	1/0/0
Disease outbreaks are a significant concern for Montana bighorn sheep	yes/no/don't know	3/1/2	4/0/2	1/0/0
Grazing domestic sheep near bighorn sheep results in bighorn sheep with disease	always/ sometimes/never	0/4/2	0/1/1	0/0/1
Bighorn sheep males transmit disease to domestic sheep	yes/no/don't know	0/1/5	0/2/4	0/0/1
Willingness to accept alternate grazing to decrease conflict with bighorn sheep management	yes/no/depends on alternatives	1/2/3	0/4/2	01/0
There is good understanding of the factors associated with disease transmission between bighorn and domestic sheep	Agree/disagree/ don't know	1/4/1	0/2/4	0/1/0
Current Montana Fish Wildlife and Parks plans for managing bighorn sheep are:	Beneficial/not beneficial/don't know	1/0/5	2/0/4	0/0/1
The impact of current MFWP bighorn sheep plans on domestic sheep	Hurt/help/no impact/don't know	4/0/1/1	3/0/0/3	0/0/0/1
Use domestic sheep for weed control	Your property	2	4	1
	Another's property	3	1	1
It is feasible to graze domestic sheep near bighorn sheep to control weeds	Yes/no	6/0	5/1	1/01
Willing to use treatment or vaccination to prevent domestic to bighorn sheep disease transmission		6	6	1

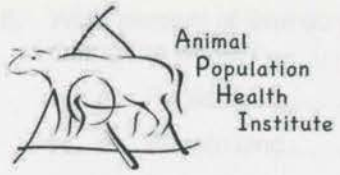
Table 5.2. Breeds of domestic sheep studied in operations at the interface with bighorn sheep and > 14.5 km from bighorn sheep.

Non-interface populations	Interface populations
Suffolk	Shetland
Targhee	Suffolk
Columbia	Targhee
Polypay	Romanov
Rambouillet	Columbia
	Romney
	Mixed breed

Table 5.3. Calculated values for the fecundity of domestic sheep operations at the interface with bighorn sheep and > 14.5 km from bighorn sheep.

		Non-interface populations	Interface populations
Proportion of replacement animals per ewe	Mean ± S.D.	0.36 ± 0.17	0.25 ± 0.11
	Range	0.18 – 0.63	0.12 – 0.47
Proportion of market lambs per ewe	Mean ± S.D.	0.83 ± 0.49	0.83 ± 0.63
	Range	0 – 1.33	0 – 1.78
Number of lambs per ewe	Mean ± S.D.	1.6 ± 0.43	1.4 ± 0.45
	Range	1.02 – 2.3	0.9 – 2.15
Proportion of weaned lambs	Mean ± S.D.	0.89 ± 0.09	0.91 ± 0.06
	Range	0.72 – 0.96	0.78 – 0.95

Appendix 1: Sheep health management questionnaire



Sheep
Health Management Questionnaire

1. Of the sheep and lambs for breeding on this operation May 1, 2005, how many were:
   - A. Ewes 1 year and older? ..... _____
   - B. Rams 1 year and older? ..... _____
   - C. Replacement lambs less than 1 year (including unweaned lambs kept for breeding? ..... _____
   - D. Market lambs less than 1 year? ..... _____

2. For the 2004 lambing season:
   - A. How many ewes were bred, if known? ..... _____
   - B. How many lambs were born, if known? ..... _____
   - C. How many lambs were weaned? ..... _____

3. In the previous 5 years, have any of your ewes had any bighorn sheep-domestic sheep hybrid offspring? ..... ☐1 Yes ☐2 Don't know ☐3 No

   If **Yes**, list how many hybrids for each year:
   - A. 2004 _____
   - B. 2003 _____
   - C. 2002 _____
   - D. 2001 _____
   - E. 2000 _____

4. How many ewes did you have in 2000, compared to this year's inventory as of May 1, 2005?
   - ☐1 No sheep in 2000
   - ☐2 Fewer sheep in 2000
   - ☐3 Same number in 2000
   - ☐4 More sheep in 2000

Questions #5-14 concern the management of your flocks during different seasons.

5. At what range of elevations do you keep your sheep during the winter? ..... _____

6. What percent of time do you keep sheep on the following types of land during the winter?

   A. Public land ..... _____
   B. Private land ..... _____
   C. Forest land ..... _____
   D. Open range ..... _____
   E. Other (specify: _____) ..... _____

7. Do you provide supplemental feed to your sheep during the winter? ..... ☐1 Yes ☐3 No
8. How do you manage your flock during the winter?

   ☐1 Herded/open range
   ☐2 Fenced range
   ☐3 Farm
   ☐4 Other (specify: _____)

9. How often do you monitor your flock during the winter?

   ☐1 One or more times per day
   ☐2 One or more times per week
   ☐3 Less than once per week

10. At what range of elevations do you keep your sheep during the summer? ..... _____

11. What percent of time do you keep sheep on the following types of land during the summer?

   A. Public land ..... _____
   B. Private land ..... _____
   C. Forest land ..... _____
   D. Open range ..... _____
   E. Other (specify: _____) ..... _____

12. Do you provide supplemental feed to your sheep during the summer? ..... ☐1 Yes ☐3 No
13. How do you manage your flock during the summer?

   ☐1 Herded/open range
   ☐2 Fenced range
   ☐3 Farm
   ☐4 Other (specify: _____)

14. How often do you monitor your flock during the summer?

   ☐1 One or more times per day
   ☐2 One or more times per week
   ☐3 Less than once per week

15. For the last completed breeding season:

    A. When did the breeding season begin? ..... _____

    B. When did the breeding season end? ..... _____

    C. When did the lambing season begin? ..... _____

    D. When did the lambing season end? ..... _____

    E. On average, how many days after lambing until lambs are weaned? ..... _____

16. During lambing, do you primarily keep the ewes:

    ☐1 On open range

    ☐2 In pasture

    ☐3 In pens

17. During the 24 hours after lambing, do you keep the ewe-lamb pairs:

    ☐1 In a separate pen by themselves

    ☐2 In a separate pen with other ewe-lamb pairs

    ☐3 With the rest of the flock

    ☐4 Other (specify: _____)

18. Do you allow visitors into sheep-raising areas? ..... ☐1 Yes ☐3 No

    If **Yes**, are any of the following required for these visitors:

    ☐1 Change boots or use boot covers

    ☐2 Restrict access to certain sheep-raising areas

    ☐3 Require that visitors have not been on another sheep operation for a specified period of time

    ☐4 Other (specify: _____)

19. In the previous 12 months, were any ewes or lambs added to this operation other than through natural additions (births)? ..... ☐1 Yes ☐3 No

    A. If **Yes**, what was the average age of the added ewes (months)? ..... _____

    B. How long ago was the last addition (ewe) made to the flock (years)? ..... _____

20. In the previous 12 months, were any rams added to this operation other than through natural additions (births)? ..... ☐1 Yes ☐3 No

    A. If **Yes**, what was the average age of the added rams (months)? ..... _____

    B. How long ago was the last addition (ram) made to the flock (years)? ..... _____

21. During 2004, did this operation:

    A. Have sheep leave for shows, exhibitions, or breeding, and return? ..... ☐1 Yes ☐3 No

    B. Graze sheep with flocks from another operation? ..... ☐1 Yes ☐3 No

    C. Have sheep that had fenceline contact with flocks from another operation? .... ☐1 Yes ☐3 No

    D. Temporarily bring rams onto the operation for breeding purposes? ..... ☐1 Yes ☐3 No

    E. Have sheep visit from another operation for any reason, such as

shearing and breeding? ☐1 Yes ☐3 No

F. Have sheep that had other contact with sheep or flocks from another operation (specify: _____) ☐1 Yes ☐3 No

22. Did you transport sheep by vehicle for any reason during 2004? ☐1 Yes ☐3 No

If **Yes**, did you use:

A. Trucks or trailers operated by a professional trucking operation? ☐1 Yes ☐3 No

B. Private trailers operated by this operation? ☐1 Yes ☐3 No

C. Other ☐1 Yes ☐3 No

23. If #22A, 22B, or 22C is **Yes**, how often were trucks and trailers disinfected before carrying your operation's sheep?

☐1 Always

☐2 Usually

☐3 Sometimes

☐4 Never

☐5 Don't know

24. During 2004, which of the following species had access to sheep-raising areas? *(Check all that apply.)*

	Fenceline contact	On pasture at different times	On pasture at same time	Other contact: sheds, holding pens, food, or water
A. Bighorn sheep	☐1	☐2	☐3	☐4
B. Rocky Mountain goats	☐1	☐2	☐3	☐4
C. Deer	☐1	☐2	☐3	☐4
D. Elk	☐1	☐2	☐3	☐4
E. Moose	☐1	☐2	☐3	☐4
F. Pronghorns	☐1	☐2	☐3	☐4
G. Bison	☐1	☐2	☐3	☐4
H. Game ranch, petting zoo, or other nondomestic hoofstock	☐1	☐2	☐3	☐4
I. Horses	☐1	☐2	☐3	☐4
J. Domestic goats	☐1	☐2	☐3	☐4
K. Cattle	☐1	☐2	☐3	☐4
L. Llamas, alpacas	☐1	☐2	☐3	☐4
M. Poultry (chickens, turkeys)	☐1	☐2	☐3	☐4

25. During 2004, did you consult with a veterinarian for any of the following reasons?

A. Disease diagnosis ☐1 Yes ☐3 No

B. Disease prevention ☐1 Yes ☐3 No

C. Information on nutrition ☐1 Yes ☐3 No

D. Production management practices ..... ☐1 Yes ☐3 No

E. Lambing problems ..... ☐1 Yes ☐3 No

G. Other (specify: _____) ..... ☐1 Yes ☐3 No

26. During 2004, for any sheep-related reason, was your operation visited by a:

A. Private practitioner (including specialists and consultants)? ..... ☐1 Yes ☐3 No

B. Federal/State veterinarian? ..... ☐1 Yes ☐3 No

C. Extension agent? ..... ☐1 Yes ☐3 No

D. Nutritionist? ..... ☐1 Yes ☐3 No

E. Other? (specify: _____) ..... ☐1 Yes ☐3 No

27. During 2004, did you vaccinate your lambs for:

A. Clostridia? ..... ☐1 Yes ☐3 No

B. Other diseases? (specify: _____) ..... ☐1 Yes ☐3 No

28. During 2004, was fecal testing done for sheep parasites? ..... ☐1 Yes ☐3 No

29. During 2004, were dewormers given to any of your sheep? ..... ☐1 Yes ☐3 No

If No, skip to #33.

30. For which reasons were dewormers given:

A. General prevention measure ..... ☐1 Yes ☐3 No

B. Because worms were seen ..... ☐1 Yes ☐3 No

C. Fecal test results indicated a need ..... ☐1 Yes ☐3 No

D. Because sheep or lambs were thin or doing poorly ..... ☐1 Yes ☐3 No

E. Other reason (specify: _____) ..... ☐1 Yes ☐3 No

31. How many times were dewormers given in 2004? ..... _____

32. If dewormers were given more than once, was more than one type of dewormer given? ..... ☐1 Yes ☐3 No

33. During 2004, did you treat your flock for external parasites? ..... ☐1 Yes ☐3 No

If **Yes**, which of the following reasons best describes why you treated your flock for external parasites:

☐1 General prevention measure

☐2 Because ectoparasites were seen

☐3 Other (specify: _____)

34. Indicate if in the previous 3 years any of the following has been present (suspected or confirmed in the flock):

A. Soremouth ..... ☐1 Yes ☐3 No

B. Diarrhea (E. coli, Vibrio, EAE) ..... ☐1 Yes ☐3 No

C. Footrot ..... ☐1 Yes ☐3 No

D.	Respiratory disease	☐1 Yes	☐3 No
E.	OPP	☐1 Yes	☐3 No
F.	Parasite (specify: _____)	☐1 Yes	☐3 No
G.	Bluetongue	☐1 Yes	☐3 No
H.	Cadeous lymphadenitis	☐1 Yes	☐3 No
I.	Dystocia due to infectious or noninfectious reasons	☐1 Yes	☐3 No
J.	Milk fever	☐1 Yes	☐3 No
K.	Grass tetany	☐1 Yes	☐3 No
L.	Copper toxicosis	☐1 Yes	☐3 No
M.	Selenium toxicosis	☐1 Yes	☐3 No
N.	White muscle (stiff lamb) disease	☐1 Yes	☐3 No
O.	Goiter	☐1 Yes	☐3 No

If all in #34 = No, skip to #37.

35. For any conditions present in #34, indicate how each was diagnosed, and whether or not you have a treatment or management plan:

		Diagnosed by:			Treatment or management plan?	
		Self	Veterinarian	Lab	Yes	No
A.	Soremouth	☐1	☐2	☐3	☐4	☐5
B.	Diarrhea (E. coli, Vibrio, EAE)	☐1	☐2	☐3	☐4	☐5
C.	Footrot	☐1	☐2	☐3	☐4	☐5
D.	Respiratory disease	☐1	☐2	☐3	☐4	☐5
E.	OPP	☐1	☐2	☐3	☐4	☐5
F.	Parasite (specify: _____)	☐1	☐2	☐3	☐4	☐5
G.	Bluetongue	☐1	☐2	☐3	☐4	☐5
H.	Cadeous lymphadenitis	☐1	☐2	☐3	☐4	☐5
I.	Dystocia due to infectious or noninfectious reasons	☐1	☐2	☐3	☐4	☐5
J.	Milk fever	☐1	☐2	☐3	☐4	☐5
K.	Grass tetany	☐1	☐2	☐3	☐4	☐5
L.	Copper toxicosis	☐1	☐2	☐3	☐4	☐5
M.	Selenium toxicosis	☐1	☐2	☐3	☐4	☐5
N.	White muscle (stiff lamb) disease	☐1	☐2	☐3	☐4	☐5
O.	Goiter	☐1	☐2	☐3	☐4	☐5

If #34D = No, skip to #38.

36. If respiratory disease was present (#34D = Yes), during which seasons did it occur?

☐1 Winter

☐2 Spring

☐3 Summer

☐4 Fall

37. Which of the following were causes of respiratory disease in the flock over the previous 3 years?

☐1 Parasites

☐2 Virus

☐3 Bacteria

☐4 Other (please specify: _____)

38. During 2004, were any animals lost to any of the following:

A. Predators? ☐1 Yes ☐3 No

B. Respiratory disease? ☐1 Yes ☐3 No

C. Nutritional disease? ☐1 Yes ☐3 No

D. Gastrointestinal disease? ☐1 Yes ☐3 No

E. Other (not respiratory, nutritional, or gastrointestinal) disease? ☐1 Yes ☐3 No

F. Bad weather? ☐1 Yes ☐3 No

39. In 2004, did you use any of the following to guard your animals:

A. Shepherds? ☐1 Yes ☐3 No

B. Llamas or alpacas? ☐1 Yes ☐3 No

C. Donkeys? ☐1 Yes ☐3 No

D. Dogs? ☐1 Yes ☐3 No

E. M-44? ☐1 Yes ☐3 No

F. Toxic collars? ☐1 Yes ☐3 No

G. Shooting? ☐1 Yes ☐3 No

H. Aerial gunning? ☐1 Yes ☐3 No

I. Fencing? ☐1 Yes ☐3 No

J. Sound devices? ☐1 Yes ☐3 No

K. Trap/snare? ☐1 Yes ☐3 No

40. Do you know of any bighorn sheep herds within 9 miles during the previous 3 years? ☐1 Yes ☐3 No

If **Yes**, list months the following animals were seen mixing with domestic sheep in this time period:

A. Adult male _____

B. Juvenile male _____

C. Female _____

D. Lambs _____

41. Do you use any strategies for keeping the following separate from your domestic sheep or grazing areas?

			If **Yes**, specify:	If **Yes**, is it effective?	
A.	Bighorn sheep	☐$_1$Yes ☐$_3$ No	_____	☐$_1$Yes	☐$_3$ No
B.	Bison	☐$_1$Yes ☐$_3$ No	_____	☐$_1$Yes	☐$_3$ No
C.	Pronghorn	☐$_1$Yes ☐$_3$ No	_____	☐$_1$Yes	☐$_3$ No
D.	Deer	☐$_1$Yes ☐$_3$ No	_____	☐$_1$Yes	☐$_3$ No
E.	Elk	☐$_1$Yes ☐$_3$ No	_____	☐$_1$Yes	☐$_3$ No

42. Have you called Montana Fish, Wildlife & Parks within the previous 3 years due to bighorn sheep contact with your flock? ☐$_1$Yes ☐$_3$ No

If No, skip to #45.

43. How long after you called about a bighorn sheep contacting your flock did it take for Montana Fish, Wildlife & Parks to respond? *(List number of times for each category.)*

A. Within a day _____

B. Within 1 to 2 days _____

C. Longer than 2 days _____

44. Were the responses in #43 satisfactory? ☐$_1$Yes ☐$_2$ Varied ☐$_3$ No

45. Do you feel that the current 9-mile buffer guidelines separating bighorn sheep and domestic sheep is an effective way to prevent disease transmission between domestic sheep and bighorn sheep? ☐$_1$Yes ☐$_2$ Don't Know ☐$_3$ No

If **No**, is the buffer:

☐$_1$ Too big

☐$_2$ Too small

☐$_3$ Other (specify: _____)

46. Do you feel that the current 9-mile buffer guidelines for keeping bighorn sheep and domestic sheep separate is affecting management of your flocks? ☐$_1$Yes ☐$_2$ Don't Know ☐$_3$ No

If **Yes**, is the effect:

☐$_1$ Harmful

☐$_2$ Beneficial

☐$_3$ Neither

47. How does the 9-mile buffer affect your management?

48. Do you feel that bighorn sheep and domestic sheep should always be kept separate from each other? ☐$_1$Yes ☐$_2$ Don't Know ☐$_3$ No

49. What do you feel is the greatest source of conflict between bighorn sheep and domestic sheep management? *(Check only one.)*

☐$_1$ No conflict exists

☐$_2$ The current 9-mile buffer decreases grazing available for domestic sheep.

☐$_3$ Disease transmission from domestic sheep to bighorn sheep

☐$_4$ Disease transmission from bighorn sheep to domestic sheep

☐$_5$ Bighorn/domestic sheep hybrids

☐$_6$ Other (specify: _____)

50. Do you feel that bighorn sheep are an important and valued part of the environment of Montana? ..... ☐$_1$Yes ☐$_2$ Don't Know ☐$_3$ No

51. Do you feel that disease outbreaks are a significant concern for maintaining bighorn sheep populations in Montana? ..... ☐$_1$Yes ☐$_2$ Don't Know ☐$_3$ No

52. Do you feel that grazing near domestic sheep results in bighorn sheep developing disease? ..... ☐$_1$ Always ☐$_2$ Sometimes ☐$_3$ Never

53. Do you feel that bighorn sheep males transmit disease to domestic sheep? ..... ☐$_1$Yes ☐$_2$ Don't Know ☐$_3$ No

    If **Yes**, specify which diseases: _____

54. Would you accept a different grazing allotment if it decreased conflict with bighorn sheep interests?

    ☐$_1$ Yes

    ☐$_2$ No

    ☐$_3$ Depends on alternatives

    ☐$_4$ Undecided

55. There is currently a good understanding of the factors involved with the transmission of disease between bighorn sheep and domestic sheep.

    ☐$_1$ Agree

    ☐$_2$ Disagree

    ☐$_3$ Don't know

56. Current Montana Fish, Wildlife & Parks plans for managing bighorn sheep are:

    ☐$_1$ Beneficial to bighorn sheep populations

    ☐$_2$ Not beneficial to bighorn sheep populations

    ☐$_3$ Don't know

57. Current Montana or Federal management plans for bighorn sheep:

    ☐$_1$ Hurt the domestic sheep industry

    ☐$_2$ Help the domestic sheep industry

    ☐$_3$ Have no impact on the domestic sheep industry

58. Do you currently graze domestic sheep to help manage or control weeds:

    A. On your property? ..... ☐$_1$Yes ☐$_3$ No

B. On another property? ..... ☐$_1$Yes ☐$_3$ No

59. Do you feel that it is feasible to graze domestic sheep near bighorn sheep for the management or control of weeds? ..... ☐$_1$Yes ☐$_3$ No

60. If there were adequate treatment or vaccination protocols for domestic sheep that would reduce or eliminate the transmission of disease between domestic sheep and bighorn sheep, would you be willing to incorporate these tools into your health management program? ..... ☐$_1$Yes ☐$_3$ No

61. Are there other management strategies which might help reduce conflict between domestic and bighorn sheep that you would consider using? *(Please list.)*

_____

CHAPTER 6

SHARED BACTERIAL AND VIRAL RESPIRATORY AGENTS IN BIGHORN (OVIS CANADENSIS) AND DOMESTIC SHEEP (OVIS ARIES) IN MONTANA

Abstract:

This study was conducted with the aim of documenting shared baseline bacteria, viruses, and parasites present in apparently healthy bighorn sheep (*Ovis canadensis*)(n = 340), domestic sheep (*O. aries*)(n = 371), and domestic goats (*Capra hircus*)(n = 45). Pasteurellaceae biovariants from retrospective studies were incorporated into analyses due to the scarcity of animals with respiratory disease in this study. A cross-sectional study for oropharyngeal bacteria and viral agents was conducted of bighorn (n = 3), domestic sheep (n = 6), and domestic goat (n = 1) populations at the bighorn/domestic sheep interface, as well as bighorn (n = 7) and domestic sheep populations (n = 6) without potential interface interactions. Domestic livestock primarily resided on private land, whereas bighorn sheep primarily resided on federal land. Few domestic sheep (n = 11 individuals) had evidence of respiratory disease, and no bighorn sheep had evidence of respiratory disease. There were 800 bighorn sheep, 1785 domestic sheep, and 355 domestic goat bacterial isolates for the uniquely identified animals that were sampled. Among these isolates, 86 different Pasteurellaceae biovariants were identified. Few (n = 19) biovariants were found only in a single species, and these constituted 3% of the total number of isolates. Biovariants associated with disease in previous chapters were isolated from both bighorn and domestic sheep in this study, but were not at a greater risk of being isolated from animals at the interface. The same biovariant was rarely recovered twice from the same individual among domestic sheep (n = 85) and goats (n = 34) resampled six months apart. *Mycoplasma* spp. was isolated for 5 of 6 domestic sheep populations and the domestic goat population, but not bighorn sheep. Antibodies to parainfluenza 3 and bovine respiratory syncytial virus were common in livestock and bighorn sheep populations, but most populations appeared to be naïve to bovine virus

diarrhea (BVD-1 and BVD-2) and infectious bovine rhinotracheitis viruses. Cluster analysis of Pasteurellaceae and viral serology results identified four different clusters ($P < 0.0001$), but these did not closely correspond to species and location categories. Nine different genera or groups of genera of endoparasites were identified in fecal samples from study animals, and included evidence of introduction of *Muelleris* spp to bighorn sheep. There was extensive sharing of agents among species, locations, and animal health classifications. This creates challenges in identifying agents and reservoirs responsible for causing disease. Further studies of multiple populations with healthy and diseased animals are required to determine whether specific agents are more common in animals with disease than in apparently healthy animals.

Key words: domestic sheep, bighorn sheep, domestic goat, Pasteurellaceae, virus, parasite, respiratory disease

Introduction

Bighorn sheep (*Ovis canadensis*) experienced substantial decreases in population numbers and range in the 19th and the early 20th century, and subsequent recovery efforts have often been limited by large scale die-offs (Buechner, 1960; Toweill & Geist, 1999; Gross *et al.*, 2000). Initial population declines were associated with settlement of western North America, and were attributed to unregulated hunting, competition for forage with domestic livestock, and disruption of historic bighorn sheep migration patterns by development. During this early period, there were die-offs of bighorn sheep that were associated with sheep scab (*Psoroptes* spp.) (Hornaday, 1901; Baillie-Grohman, 1902).

Subsequent respiratory disease die-offs in the middle and early 20th century were primarily associated with lungworm (*Protostrongylus* spp.)(Pillmore, 1958). There is currently a focus on pneumonic pasteuerellosis as a cause of bighorn sheep die-offs (Onderka *et al.*, 1988; Foreyt *et al.*, 1994; Dassanayake *et al.*, 2009). Additional hypotheses for causes of bighorn sheep die-offs include environmental stressors (Spraker *et al.*, 1984) and forage selenium deficiencies (Dean *et al.*, 2002; Hnilicka *et al.*, 2002). Identification of the cause of bighorn sheep die-offs is important for identifying potential preventive management strategies.

Pasteurellosis has been considered as both an opportunistic and a primary pathogen disease in domestic and wild animals (Miller, 2001). A commonly accepted model of pasteurellosis in domestic ruminants is as an opportunistic, endogenous bacterial infection that is the consequence of environmental and host conditions that favor the development of disease following pulmonary colonization by the bacteria (Yates, 1982; Czuprynski *et al.*, 2004; Zecchinon *et al.*, 2005; Dabo *et al.*, 2008). This model emerged following the recognition that Pasteurellaceae are a normal part of animal's oropharyngeal microflora, in combination with a lack of concordance among experiments that pursued single agent hypotheses. Nonetheless, respiratory disease, and pasteurellosis in particular, remain important causes of loss to the domestic sheep industry (USDA, 2001a; Pugh, 2002)

Early reports suggested that pasteurellosis was an opportunistic infection in free-ranging bighorn sheep (Evans, 1937; Marsh, 1938). More recent in vitro and whole animal studies under captive conditions have suggested that bighorn sheep are inherently susceptible to pasteurellosis, particularly to domestic sheep strains (Onderka *et al.*, 1988; Foreyt *et al.*, 1994; Dassanayake *et al.*, 2008; Dassanayake *et al.*, 2009). This has lead to

land use management policies that include the use of 14.5 km buffers to keep bighorn and domestic sheep populations separate (Unites States Department of the Interior, 1998). However the actual risk of free-ranging bighorn sheep developing spontaneous pasteurellosis or as a result of contact with domestic sheep is uncertain, due to the scarcity of baseline data and the practical challenges of documenting the causes of die-offs under field conditions. This uncertainty has resulted in contention over land use policy at the bighorn/domestic sheep interface (United States Geologic Survey/Bureau of Reclamation Office, 2006), particularly as both interest groups have an interest in reversing historic population declines (Buechner, 1960; Lupton, 2008). Consequently, conflict between these interest groups is likely to continue. Although much of this contention reflects differences in values and other sociological concerns, addressing the biological concerns that exist may lead to improved domestic and bighorn sheep management strategies.

Pasteurellaceae species commonly associated with respiratory disease outbreaks in bighorn and domestic sheep are *Mannheimia* (*Pasteuerella*) *haemolytica* (Angen *et al.*, 1999), *P.* (*Bibersteinia*) *trehalosi* (formerly *P. haemolytica* biotype T)(Sneath & Stevens, 1990; Blackall *et al.*, 2007), and *Pasteurella multocida* (Miller, 2001; Weiser *et al.*, 2003; Watson & Davies, 2002; Odugbo *et al.*, 2004; George *et al.*, 2008b). These species represent a heterogenous mix of bacterial strains that can be responsible for a range of clinical signs. Consequently, further distinction of strains within a species is desirable for epidemiological studies.

Subclassification of *Pasteuerella*, *Bibersteinia*, and *Mannheima* (P/M) species based on capsular antigens has been the basis of a serotype classification scheme that assigns isolates to biogroups (Confer, 1993; Blackall *et al.*, 2007). However, cross-

agglutination or non-reactions with typing sera prevent classification of some isolates using this scheme, particularly for wildlife isolates (Jaworski *et al.*, 1998). Consequently, a biovariant classification scheme based on microbiological characteristics and biochemical utilization tests of isolates in culture was established (Bisgaard & Mutters, 1986; Jaworski *et al.*, 1998). The biovariant classification scheme distinguishes among a greater number of P/M strains than do biogroups.

The isolation of P/M from bighorn and domestic sheep without signs of disease (Dunbar *et al.*, 1990; Queen *et al.*, 1994; Ward *et al.*, 1997; Jaworski *et al.*, 1998) suggests that a simple agent exposure:disease relationship does not exist, or that only some strains of P/M cause disease. In addition, these species may share P/M microflora. Sharing of P/M among these species is apparent using both the biogroup and the biovariant classification schemes (Tomassini *et al.*, 2009). Evidence for the presence of *Mycoplasma* spp., viral agents, and parasites in cases where P/M are isolated further complicates interpretation of the role of P/M in bighorn and domestic sheep respiratory disease (Aune *et al.*, 1998; Brogden *et al.*, 1998; Pugh, 2002; Rudolph *et al.*, 2007; Besser *et al.*, 2008). Even where P/M are identified in sympatric domestic livestock and bighorn sheep, the reservoir and direction of possible transmission are uncertain, as there appears to be interspecies sharing of of P/M without the occurrence of disease (Ward *et al.*, 1997; Rudolph *et al.*, 2003; Tomassini *et al.*, 2009). Consequently, it is not possible to distinguish among P/M that are associated with disease or a particular species without baseline data for multiple populations of bighorn and domestic sheep.

The aim of this chapter is to evaluate bighorn and domestic sheep for evidence of shared agents with presumed pathogenic potential. This was considered using several lines of evidence. Data was evaluated to identify Pasteurellaceae biovariants,

Mycoplasma spp., viral agents, and parasitic agents that were present in > 1 species. In addition, due to the dearth of animals with apparent respiratory disease, Pasteurellaceae biovariants that were associated with bighorn and domestic sheep with respiratory disease in Chapters 3 and 4 were evaluated for their presence in healthy populations. Pasteuerellaceae that were identified only in interface animals of one species were evaluated for evidence that these biovariants were common in the sympatric species, as a preliminary means of identifying potential reservoirs of infection. Cluster analysis was conducted to determine whether populations near to and > 14.5 km from the interface had characteristic Pasteurellaceae and viral exposure. This was a means of identifying associations between agents and categories of host species and location relative to the bighorn/domestic sheep interface. Secondary objectives included consideration of post-mortem data on two bighorn sheep and agents in a goat population that was co-managed with a domestic sheep population at the wildlife/domestic animal interface. In addition, individual animals in three domestic sheep and one goat population were resampled for Pasteuerellaceae at a six month interval as a means of assessing temporal variation of Pasteurellaceae microflora isolates.

Methods

This study sampled four different types of populations: A. bighorn sheep populations without domestic sheep known to exist within 14.5 km (9 miles)(n = 7), B. domestic sheep populations without bighorn sheep known to exist within 14.5 km (n = 4), or for which contact between populations was not possible due to physical separation by housing development (n = 1) or season (n = 1), C. interface bighorn sheep populations with domestic sheep known to exist within 14.5 km (n = 3), D. interface domestic sheep

populations with bighorn sheep known to exist within 14.5 km (n = 6). For each interface bighorn sheep population, two domestic sheep populations were identified as 'pairs' for the purpose of attempting to identify shared agents. One goat population that was co-managed with an interface domestic sheep population was included in the study. The choice of 14.5 km distance was selected based on management guidelines for bighorn and domestic sheep (Unites States Department of the Interior, 1998). The proximity of bighorn sheep to livestock populations at the interface was confirmed by communications from producers (Chapter 5). Populations were opportunistically sampled based on location and bighorn sheep management activities or domestic operator willingness to participate. Population identification was coded due to participant confidentiality concerns. Locations for populations were recorded in WGS 84 GPS format. Non-interface bighorn sheep populations included Thompson Falls (N47.58050 W115.24275), Parma/Plains (N 47.23140 W114.48014), Sun River (N47.36118 W112.45391), Charles M. Russell National Wildlife Refuge (N45.12583 W112.36854), National Bison Range (N 47.36673 W 114.25492), Glacier National Park (N48.43282 W113.44495), and Harper's Ferry (N47.67228 W -107.95405). Interface bighorn sheep were sampled from populations near Winifred, MT (N47.55967 W109.37517), and Anaconda, MT (N45.64884 W112.68929), as well as from the Sleeping Giant bighorn sheep population (N46.97881 W112.00734). Research protocols were approved by Colorado State University Institutional Animal Care and Use Committee protocol number ACUC 05-05-283A-01.

Bighorn sheep population characteristics were compiled from winter aerial surveys conducted by Montana Fish Wildlife and Parks (MFWP) in 2003. Domestic livestock population characteristics were compiled from questionnaires that were based on

previous NAHMS (USDA, National Animal Health Monitoring System) questionnaires (USDA, 2001a; USDA, 2001b; USDA, 2002; USDA, 2003), which were verbally administered after biological samples were collected (Chapter 5).

Bighorn sheep were captured 2004 -2006. Most were captured by helicopter net gunning during the months of December - March, followed by hobbling and blindfolding for transport to animal processing sites. Chemical restraint was used for bighorn sheep from three populations: Anaconda (n = 25), Glacier National Park (n = 61), and National Bison Range (n = 10). All animals had ear tags or radio collars applied at the time of processing for individual identification. Physical examination and biomedical sample collection of bighorn sheep was conducted as quickly as possible to minimize overheating and capture stress. Snow, water, or ethanol was applied to individuals to correct hyperthermia, as needed. Animals were either released at the capture site or transferred to trailers for transport to translocation release sites.

Domestic livestock were manually restrained for physical examinations and biomedical sample collection during the spring or fall of 2005- 2006. All animals were individually identified with ear tags. Procedures were conducted quickly to minimize overheating and distress. All domestic sheep and goats were released to their populations upon completion of sampling. Physical examination data of animals in the study included observations of respiratory disease (nasal discharge or coughing).

Oropharyngeal microflora were sampled following fixation of the mandible in a "mouth open" position with a mouth gag that had been disinfected or with clean gloved hands (Drew *et al.*, 2005). Swabs that contacted the tongue, teeth, or other potential sites of contamination were discarded and the process was repeated until a sample representative of the oropharyngeal flora was collected. Two sterile dacron swabs were

used to swab the tonsils and surrounding oropharyngeal region using methods developed for bighorn sheep (Drew *et al.*, 2005), placed in sterile media tubes containing modified Cary Blair media (Port-a-cul, , Becton-Dickinson, Franklin Lakes, New Jersey, 07417 USA), and shipped chilled without freezing to a reference laboratory (University of Idaho, Caine Veterinary Teaching Center, Caldwell, Idaho 83607, USA)(CVTC) for Pasteurellacea spp. and *Mycoplasma* spp. culture within 72 hours of collection. Individuals from three domestic sheep populations and a goat population co-managed with one sheep population were sampled twice, six months apart as a means of assessing temporal stability of oral microflora.

Blood for serology was collected into sterile serum collection tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, New Jersey, 07417 USA), kept cool, centrifuged, serum separated, and hand carried or shipped frozen to a veterinary diagnostic laboratory (Montana Veterinary Diagnostic Laboratory, Bozeman, Montana, 59771 USA)(MVDL) for viral serology.

Feces were taken from the rectum or upon defecation during processing, kept chilled, and submitted to the veterinary diagnostic laboratory for fecal floatation and Baermann analyses (Beane & Hobbs, 1983; Hoar, 1995).

All biological samples collected from all populations were handled as unique samples identified by date and individual. Due to processing, shipping, financial, and biological (ie. animals without feces) reasons, not all animals had complete results for all agents analyzed.

Bacterial culture procedures

At CVTC, one oropharyngeal swab from each animal was inoculated onto nonselective Columbia blood agar (CBA), (Becton Dickinson & Co., Sparks, Maryland 21152, USA) containing 5% sheep blood, and CBA with selective antibiotics for Pasteurellaceae, containing 5% bovine blood (Jaworski *et al.*, 1993), and incubated for 18 to 24 hr at 37°C in a 10% CO_2 atmosphere. Following incubation, representatives of each colony type were propagated on fresh CBA for species and biovariant classification.

Species and biovariant classification of Pasteurellaceae isolates

Isolates were determined to be *M. haemolytica* or *P.* (*B*). *trehalosi*, as opposed to *P. multocida*, based on the following characteristics: urea- and indole-negative; oxidase-, nitrate-, glucose-, sucrose, and mannitol-positive; and failure to grow or poor growth on MacConkey's agar. *Mannheimia haemolytica* and *P.* (*B*). *trehalosi* were further distinguished if they were trehalose-negative-or trehalose-positive, respectively. Biovariant classification was done using a modification of a biochemical testing system developed for isolates from domestic animals (Bisgaard and Mutters, 1986), adapted for identifying isolates from wildlife (Jaworskiet al., 1998). Briefly, the wildlife method uses the results from 23 microbiological characteristics and biochemical utilization tests to separate isolates into biovariants which are hierarchically classified based on species, type, and exceptions. In addition, isolates with zones of hemolysis on blood agar were classified as beta-hemolytic. Following speciation and biovariant classification, isolates were stored frozen at -70 C in phosphate-buffered saline: glycerol (4:6 v/v, pH 7.2).

Mycoplasma identification

At CVTC, the second oropharyngeal swab was placed in *Mycoplasma* broth and incubated at 37° C for 36 – 48 hours (Atlas, 1993). Broth was subsequently streaked on *Mycoplasma* plates and incubated at 37°C with 5 – 10% CO_2 for 5 – 7 days. Finally, *Mycoplasma* colonies were selected and plated on fresh medium. Due to financial constraints, it was not possible to assay every animal's swabs for *Mycoplasma*, although every population had a minimum of 5 samples cultured for *Mycoplasma*.

Tissue samples

Tissue samples were available from two bighorn sheep that were euthanized during the study using American Veterinary Medical Association approved procedures (Beaver *et al.*, 2001). One 9 y female (#3007) from the Malta population was euthanized due to capture related injuries that were too severe to warrant release. Oral swab (from processing protocols), tonsilar tissue, and lung samples were submitted for Pasteuerellaceae and *Mycoplasma* culture, and lung samples were submitted in formalin for histopathology to MVDL. A second (male) bighorn sheep was residing in animal shelters with domestic sheep and goats for many months. This individual was euthanized as a part of Montana Fish Wildlife and Parks policy. This policy is intended to prevent bighorn sheep that contact domestic sheep from serving as vectors for novel agents upon returning to native populations. Oral swab and lung tissue from this individual were submitted for Pasteuerellaceae and *Mycoplasma* culture.

Serology procedures

Serology was conducted at MVDL for viruses with the potential to cause or predispose animals to respiratory infection. A microtiter serum neutralization (SN) test was used to detect antibodies to infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea (BVD I and BVD 2), and bovine respiratory syncytial virus (BRSV)(Fenner *et al.*, 1993; Cottral, 1978; Center for Veterinary Biologics & National Veterinary Service Laboratories, 1998). A hemagglutination inhibition test was used to detect antibodies to parainfluenza-3 (PI-3)(Tortora *et al.*, 1992; Fenner *et al.*, 1993; Worley *et al.*, 1988). Animals with serology results ≥ 8 were classified as positive for antibodies to IBR, BVD I and 2, BRSV, and PI-3. Seroconversion was defined as a ≥ 4 fold increase in titer for any of the four viruses

Fecal parasitology

Fecal samples were analyzed by a parasitologist at the MVDL using conventional fecal floatation and Baermann assay methods (Worley *et al.*, 1988; Hoar, 1995). Conventional semi-quantitative Baermann assays results have not been associated with meaningful biological processes and financial constraints limited the number of fecal samples that could be analyzed. Consequently, only the presence of parasites is reported.

Data Analyses

Data were manually entered from laboratory reports into a Microsoft Access database (Microsoft Corporation, One Microsoft Way, Redmond, WA 98052 USA) or imported using laboratory created electronic files. Data from this study and retrospective studies (Chapters 3 and 4) were included in the same database and were combined for

some analyses. Data were directly obtained from the database or exported into Microsoft Excel files for descriptive data and tables. Data were exported from the database into SAS (SAS Institute Inc., Cary, North Carolina, 27513 USA) files for statistical analyses. Individuals of each host species were classified as positive or negative for specific agents, based on the results of bacterial culture, viral serology, and fecal parasitology. When there was data from more than one sample event available for an individual, data from the first sampling event was used, except where temporal comparisons were conducted.

Potential Transmission

Evidence for Pasteurellaceae transmission across the bighorn/domestic sheep interface was qualitatively evaluated in both directions by identifying biovariants that were present only in interface populations of one species. Provisional evidence for such transmission was considered to exist if the biovariant was subjectively considered common in adjacent populations of the sympatric species: Pasteurellaceae that were present only in interface populations of bighorn sheep were assessed for their presence in adjacent domestic sheep populations, and Pasteurellaceae that were present only in interface populations of domestic sheep were assessed for their presence in adjacent bighorn sheep populations.

Temporal variability

Pasteurellaceae isolates for individuals that were resampled at a six month interval in domestic sheep populations (n = 3) and the goat population were compared for their occurrence at both sampling events. Each biovariant isolated from an individual was considered an isolate event. The sum of isolate events for each individual at both sample

events, less those identified twice in the same individual, was used as a measure of instances where a biovariant could be isolated ≥ 1occasion in the same individual.

Statistical analyses

All statistical analyses were conducted using SAS version 9.2. All Chi-square analyses were conducted using the FREQ procedure with the threshold for significance set at a value of $P \leq 0.05$. Chi-square tests assume independence of data.

Separate Chi-square analyses were conducted for each species's Pasteurellaceae isolates. Chi-square analyses were conducted to determine whether there was an association between the isolate being collected at the bighorn/domestic sheep interface and whether the isolate was beta-hemolytic. Chi-square (2×2) analyses were also conducted to determine whether there was an association between the isolate being collected at the bighorn/domestic sheep interface and whether the isolate had been associated with animals with respiratory disease in previous studies (Chapters 3 and 4). For these analyses, isolates were classified as "healthy" unless previously associated with an animal of the same species with respiratory disease ("diseased"). Isolates were classified as "healthy" or "diseased" for each instance that the isolate was observed in these analyses.

K-means cluster analysis using the FASTCLUS procedure was conducted to classify agents that were characteristic of each species at and distant to the the bighorn/domestic sheep interface. The variables used in the cluster analysis were each animal's exposure to potential respiratory agents, on a presence:absence basis. The agents considered were each of the Pasteurellaceae biovariants isolated from the individual animal, as well as the individual's serologic evidence for antibodies to respiratory viruses

(PI-3, BRSV, BVD-1. BVD-2, IBR). The result of the analyses assigned animals to one of four clusters. The cluster assignment was cross-classified with species-location in a 4 × 4 table and tested for association using a Chi-square test. The four species-location categories of interest were: 1. bighorn sheep populations > 14.5 km distant to the interface, 2. bighorn sheep populations at the interface, 3. domestic sheep populations at the interface, and 4. domestic sheep populations > 14.5 km distant to the bighorn/domestic sheep interface.

Results

A range of population sizes was sampled for bighorn and domestic sheep (Table 6.1). The number of animals sampled in each population varied due to availability and cost constraints. Bighorn sheep primarily inhabited federal land, whereas domestic sheep were primarily on private land. Domestic sheep with evidence of respiratory disease (n = 11) were in four interface populations (n = 10) and one noninterface population (n = 1). No goats or bighorn sheep had evidence of respiratory disease.

There were 800 bacterial isolates from bighorn sheep, 1785 isolates from domestic sheep, and 355 isolates from domestic goats, with 88 different bacterial strains identified to at least the species level (Table 6.2). Among these isolates, 86 different Pasteurellaceae biovariants were identified. Thirty-six biovariants were identified in a sample of 340 bighorn sheep, 72 biovariants were identified in a sample of 371 domestic sheep, and 27 biovariants were identified in a sample of 45 goats. Few (n = 19) biovariants were found only in a single species, and these constituted 3% of the total number of isolates. One hundred seventy bacterial isolates were not identifiable by species.

Fifty-eight biovariants and bacterial species that were previously identified in a retrospective study of bighorn sheep (Chapter 3) were not identified in bighorn sheep in this study, and six bighorn sheep biovariants that were isolated in this study were not identified in that retrospective study (Table 6.3). Fifty-two biovariants and bacterial species that were previously identified in a retrospective study of domestic sheep (Chapter 4) were not identified in domestic sheep in this study, and thirteen biovariants of domestic sheep that were isolated in this study were not identified in the retrospective study (Table 6.3).

Potential Transmission

Fourteen Pasteurellaceae biovariants were identified only in interface populations of bighorn sheep, (Table 6.2), and these accounted for 6% of the total bighorn sheep isolates. Only 50% of these biovariants were also identified in domestic livestock populations, and only four (*M. hemolytica* 1^{G}, *M. hemolytica* $U^{\alpha\beta}$, *P. multocida* U6, and *P.* (*B.*) *trehalosi* 11^{E}) were identified in bighorn and domestic sheep populations that were at the same interface. In noninterface populations of bighorn sheep, nine Pasteurellaceae biovariants were identified only.

Twelve Pasteurellaceae biovariants were identified only in interface populations of domestic sheep, (Table 6.2), and these accounted for 3% of the total domestic sheep isolates. Only five of these biovariants (*M. hemolytica* $U^{\beta B}$, *P. multocida* A, *P. multocida* U6, and *P.* (*B.*) *trehalosi* 2^{B}) were also identified in bighorn sheep populations that were at the same interface. In noninterface populations of domestic sheep, nineteen Pasteurellaceae biovariants were identified only.

Disease

Each of the Pasteurellacea biovariants previously associated with bighorn sheep classified as diseased (Chapter 3) was also identified in healthy bighorn sheep in this study or the previous study, with the exception of isolates for *M. haemolytica* $16^{\alpha E}$ (n = 1) and *P.* (*B.*) *trehalosi* 2^{CDS} (n = 2)(Table 6.2). Each of these bighorn sheep biovariants (Chapter 3) was also identified in apparently healthy domestic sheep in this study, or the previous study, with the exceptions of *P.* (*B.*) *trehalosi* 2^{BG} (n = 1) and *P.* (*B.*) *trehalosi* 4^{B} (n = 7). In addition, each of these bighorn sheep biovariants was isolated from domestic sheep classified as diseased (Chapter 4), except for *M. haemolytica* $U^{\beta BEX}$ (n = 4), *P.* (*B.*) *trehalosi* 2^{BG} (n = 1), and *P.* (*B.*) *trehalosi* 4^{B} (n = 7).

Each of the Pasteurellacea biovariants previously associated with domestic sheep classified as diseased (Chapter 4) was also identified in apparently healthy domestic sheep in this study or previously, with the exception of isolates for *M. haemolytica* 1^{B} (n = 3) and *P.* (*B.*) *trehalosi* 2^{CD} (n = 6) (Table 6.2). Of these domestic sheep biovariants classified as diseased in this study and Chapter 4, 56% were also isolated from apparently healthy bighorn sheep and 23% were isolated from bighorn sheep classified as diseased.

There was not a significant association between whether an isolate was classified as diseased and whether the isolate was collected at the interface for bighorn sheep (P = 0.32; OR 0.83, 95% 0.58 – 1.20). For domestic sheep, the odds of an isolate classified as diseased being distant to the interface was estimated to be 1.31 (95% CI 1.08 – 1.60; P = 0.0073) times the odds of being associated with disease at the interface.

There was not a significant association between whether an isolate was beta-hemolytic and whether the isolate was collected at the interface for domestic (P = 0.89;

OR 0.98, 95% CI 0.71 – 1.34) or bighorn sheep populations (P = 0.41; OR 0.76, 95% CI 0.40 – 1.45).

Euthanized bighorn sheep

A male bighorn sheep euthanized for closely associating with domestic sheep and goats had no apparent clinical abnormalities. There were more Pasteurellaceae biovariants from an oral swab (n = 4) than from lung tissue (n = 2) (Table 6.5). There were two biovariants (*P.* (*B.*) *trehalosi* 2^{CDS} and *P.* (*B.*) *trehalosi* 4^{CDS}) isolated from this male that were not identified in the closest bighorn sheep population, but were identified in the sympatric domestic livestock. All samples from this male were negative for *Mycoplasma* spp., although the sympatric goats and domestic sheep populations had Mycoplasma spp. present.

A bighorn sheep female euthanized due to capture related injuries was estimated to be 9 y old. Both oral swab and tonsil samples had isolates of *P.* (*B.*) *trehalosi* 2^{b}, *P.* (*B.*) *trehalosi* 2^{be}, and *Streptococcus* spp. (Table 6.4). *Bacillus* spp. was also isolated from the oral swab. There were no bacterial isolates from lung tissue, and *Mycoplasma* cultures did not result in isolates. Histopathology of lung tissue indicated verminous pneumonia due to presumptive *Protostrongylus* spp. infection.

Temporal variation

Individuals (n = 119) from four domestic livestock populations were resampled for Pasteurellaceae (Table 6.6). Biovariants were isolated from the same individual at both sample events at 5% of the potential isolate events for domestic sheep and 4% for

domestic goats. None of the domestic sheep and goats sampled twice had complete concordance in the biovariants identified for each sampling period.

Bacteriology - *Mycoplasma*

Mycoplasma was isolated from 92% of domestic sheep populations and the goat population, but not from bighorn sheep (Table 6.7). *Mycoplasma* was isolated from > 66% of domestic sheep, and from 22% of domestic goats. *Mycoplasma* was isolated from one domestic sheep with evidence of respiratory disease.

Virology

Every population tested had serologic evidence of PI-3 virus (Table 6.8). All populations except for non-interface populations of domestic sheep (n = 1) and bighorn sheep (n =3) had serologic evidence for BRSV. Five individuals in two domestic sheep populations had serologic evidence for both BVD-1 and BVD-2 and all titers were < 128. Two bighorn sheep and one goat had low titers (8) to IBR, and the goat population was at the same interface as one of the bighorn sheep. Of the domestic sheep (n = 85) in three populations and domestic goats (n = 34) in one population that were sampled six months apart, there was evidence for seroconversion to PI-3 (n = 26) and BRSV (n = 5). For domestic sheep with signs of respiratory disease (n = 11), there was evidence for antibodies to PI-3 (n = 9) and BRSV (n = 5), but not BVD-1, BVD-2, or IBR.

Cluster analysis

Cluster analyses of individual's Pasteurellaceae and serology results (Table 6.9) indicated a significant association between cluster classification and species-location

categories ($P < 0.0001$). There was an overrepresentation of domestic sheep in cluster 4, an overrepresentation of bighorn and domestic sheep at the interface for cluster 1, and an underrepresentation of non-interface populations for cluster 1. However, the clusters with the highest and lowest percentages for each row overlapped and did not clearly segregate among species-location categories.

Parasitology

Nine different genera or groups of genera were identified in fecal samples from study animals (n = 355) (Table 6.10). *Muelleris* spp. and *Protostrongylus* spp. were concurrently present in three noninterface bighorn sheep populations.

Discussion

This study was conducted as a pilot project for understanding the dynamics of agents potentially transmitted in either direction at the bighorn sheep/domestic livestock interface. There is currently uncertainty over the causes and management options for respiratory disease outbreaks in bighorn sheep. Respiratory disease, and in particular, pasteurellosis, are also concerns of the domestic sheep industry (USDA, 2001a; Pugh, 2002). The aim of this chapter is to evaluate bighorn and domestic sheep for evidence of shared agents with presumed pathogenic potential. Due to the controversy regarding interspecies transmission of agents that cause disease, inferences on the potential for the development of disease and transmission are also presented.

Two previous studies in Nevada and California that examined fewer populations have compared the baseline Pasteurellaceae of sympatric bighorn and domestic sheep

(Ward *et al.*, 1997; Tomassini *et al.*, 2009). These studies did not identify Pasteurellaceae that appeared to be associated with bighorn sheep respiratory disease.

Most previous research on bighorn sheep respiratory disease has been limited to experimental work under controlled conditions (Onderka *et al.*, 1988; Foreyt *et al.*, 1994; Dassanayake *et al.*, 2009) or post-mortem data collected during or after an outbreak (Cassirer *et al.*, 1996; Rudolph *et al.*, 2003; Rudolph *et al.*, 2007). The former are limited by uncertainty as to the frequency and magnitude that laboratory findings are applicable to field settings, and the latter are limited by a dearth of baseline comparisons with healthy animals. Similarly, case reports of outbreaks of pasteurellosis in domestic sheep have limited inference without baseline comparisons (Mishra *et al.*, 2000; Watson & Davies, 2002; Odugbo *et al.*, 2004). Consequently, this study contributes to the need for more baseline data on the agents associated with disease in bighorn and domestic sheep.

Populations with a range of sizes (Table 6.1) were sampled opportunistically based on agency or collaborator activities for bighorn sheep, and livestock operator's willingness to participate. Consequently, based on standards for observational studies (Levy & Lemeshow, 1991; Dohoo *et al.*, 2003), the potential for extrapolating the inferences from this opportunistic study to other populations and locations is limited. In addition, few males were sampled in this study. Nevertheless, these results provide a baseline of agents present in largely healthy domestic livestock and bighorn sheep in Montana.

Bighorn sheep in this study resided primarily on federal land, and domestic livestock populations primarily resided on private property (Table 6.1). This indicates that there is the potential for conflict at the boundaries of private property and federal

lands when there is discordance in the management objectives for bighorn and domestic sheep.

It was not possible to sample all populations at these interfaces. It is, therefore, not possible to address all possible routes of agent transmission between these species. In addition, it is not possible to address interactions with other species of wildlife or domestic species.

Evidence of respiratory disease was limited to mild signs in 11 domestic sheep and there was little variation in physical examination findings. Consequently, this study focused on identifying shared agents, rather than identification of risk factors associated with disease. However, due to the need to provide preliminary data on agents with pathogenic potential, the limitations to the data prompted inclusion of data from retrospective studies of Pasteurellaceae biovariants associated with bighorn (Chapter 3) and domestic sheep (Chapter 4) with signs of respiratory disease.

Pasteurellaceae

This study identified a large number (n = 86) of different Pasteurellaceae biovariants in largely healthy bighorn sheep and domestic livestock in Montana (Table 6.2). Many biovariants were uncommon. Many (n = 110) biovariants were not identified in both this study and the retrospective studies (Chapters 3 and 4) (Table 6.3). Most biovariants were identified in multiple species. Only 19 biovariants, constituting 3% of the total number of isolates in this study, were identified in only a single species,when this study's results are combined with the results of the retrospective studies. It is possible that more extensive sampling would associate all biovariants with multiple species. Therefore, although some biovariants appear to be more commonly associated

with a host species, apparently healthy bighorn sheep and domestic livestock share many Pasteurellaceae biovariants.

Potential transmission

The use of the concept of "contact" was avoided for this study, although interspecific transmission of pathogenic agents to naïve populations is of interest. This is due to the inability to define and measure this variable for bighorn and domestic sheep under field conditions. Consequently, the 14.5 km buffer established for land management purposes (Unites States Department of the Interior, 1998) was used as a practical and management-based approach. It was supported by interviews and observations that indicated that bighorn sheep were in visual or close contact with domestic sheep operations classified as interface populations. At each of the three bighorn/domestic sheep interfaces in this study, two domestic sheep populations and one bighorn sheep population that shared the same interface were sampled. These proximate domestic and bighorn sheep populations were used for identifying instances of interspecific Pasteuerellaceae transmission.

A qualitative assessment of Pasteurellaceae transmission was conducted. This was done by identifying biovariants that were present only in interface populations of one species and in adjacent populations of the sympatric species. This assumes that if a biovariant is common in one species and is only seen in interface populations of the other species, transmission from the "common" reservoir to the sympatric species at the interface may have occurred. It also assumes that transmission at the bighorn/domestic sheep interface is the only explanation for this observation. Consequently, this assessment is provisional, inferences are tenuous, and further research is required to

support any conclusions. If these limitations are accepted, only nine such biovariants were found in bighorn and domestic sheep populations that were in proximity at the same interface. For perspective, 28 biovariants were found only in noninterface populations. Therefore, it is possible that the appearance of some biovariants exclusively in interface populations of a species is more a reflection of few populations being sampled for uncommon biovariants than of a true biological phenomenon. Regardless, this data is consistent with infrequent transmission of Pasteurellaceae at the bighorn/domestic sheep interfaces in this study. If this is generally applicable to bighorn/domestic sheep interfaces and if interspecific transmission results in disease, infrequent transmission is consistent with sporadic outbreaks of respiratory disease.

Transmission – cohabitating bighorn sheep male Pasteurellaceae

Necropsy results were available for this study from an apparently healthy bighorn sheep male that had inhabited facilities with apparently healthy domestic sheep and goats for several months. This provided an opportunity to investigate a known instance where a bighorn sheep was in close proximity with domestic sheep and goats. This male was well within a distance (18.3 m) of domestic sheep for airborne transmission of viable Pasteurellaceae (Dixon *et al.*, 2002), and interspecies nose-to-nose contact or contact with food and water containing domestic livestock saliva was likely. *Pasteurella* (*B.*) *trehalosi* 2^{B} was the most common biovariant isolated from bighorn sheep (n = 245) in this study, and was isolated from the euthanized male and one sympatric domestic sheep (Table 6.5). In the opposite direction of potential transmission, isolates (*P.* (*B.*) *trehalosi* 2^{CDS} and *P.* (*B.*) *trehalosi* 4^{CDS}) that had not been identified in nearby bighorn sheep but were identified in proximate domestic sheep and goats were isolated from the euthanized male.

Further study using DNA fingerprinting technology would be needed to confirm the similarity of these isolates, but would not confirm transmission or the direction of transmission. In addition, additional cases under field conditions are needed to determine whether these observations represent a general phenomenon. Furthermore, even if transmission is demonstrated, there is a need to determine the risk of developing disease due to such transmission.

Although the euthanized male appeared to be clinically healthy, Pasteurellaceae were isolated from his pulmonary tissue. In domestic animal models of pasteurellosis, colonization of lungs occurs in states of pulmonary disease (Ackermann & Brogden, 2000). Histopathology was not available to clarify whether subclinical pulmonary pathology was present. While further data is needed on this point, it is consistent with concerns that clinically normal bighorn sheep that closely associate with domestic livestock can acquire novel infections and subsequently transmit these infections to naïve populations of bighorn sheep. It has been hypothesized that this can result in outbreaks of disease. Consequently, policies for removing such individuals may be a prudent, precautionary means of minimizing the odds of outbreaks of respiratory disease occurring.

Disease

There is concern that populations located at the bighorn/domestic sheep interface are at a greater risk for exposure to pathogenic Pasteurellaceae. Inferences from this study on the potential to develop disease are limited, as only a few (n = 11) domestic sheep had mild signs of respiratory disease. All biovariants associated with animals previously (Chapters 3 and 4) classified as diseased were also found in healthy animals of the same

species, with the exception of four biovariants constituting a total of 12 isolates in this study (Table 6.2). Furthermore, these biovariants were also generally identified in apparently healthy and clinically diseased animals of the sympatric *Ovis* species. This ubiquity suggests that there is not an invariant relationship between the presence of Pasteurellaceae biovariants and the presence of clinical disease. This presents challenges for indentifying increased risks for disease at the bighorn/domestic sheep interface.

A preliminary assessment for the risk of disease at the interface due to pathogenic Pasteurellaceae was conducted by incorporating data from retrospective studies. This was accomplished by classifying biovariants that were associated with animals with respiratory disease in retrospective studies as diseased, and all others as healthy. The biovariant's health classification was compared to whether the isolate came from an interface population using a 2 × 2 Chi-square table analysis. This analysis was conducted separately for each species. For bighorn sheep, the relationship was non-significant. For domestic sheep, the relationship was significant, although the increased odds of being classified as diseased were for animals distant to the interface. Consequently, these data do not indicate an increased risk of exposure to pathogenic Pasteurellaceae at the interface. These results could be due to the characteristics of the few populations that were studied, imprecision in classifying isolates in the retrospective studies, the challenges of accurately defining interface populations as a proxy for contact, or other factors.

A second 2 × 2 Chi-square table analysis was conducted for evidence of an increased risk of pathogenic Pasteurellaceae at the bighorn/domestic sheep interface. This analysis tested for an association between Pasteurellaceae with beta-hemolytic characteristics and whether these biovariants were collected near to or far from the

interface. Beta-hemolysis is sometimes used as an index of microbial pathogenicity, and there is some evidence that beta-hemolytic Pasteurellaceae may be associated with pathogenicity in bighorn sheep (Chapter 3). However, there was not an association between location at the interface and isolate beta-hemolysis for either bighorn or domestic sheep. It is possible that beta-hemolysis is not an appropriate index for Pasteurellaceae pathogenicity, and that as yet unidentified characteristics of the Pasteurellaceae would be better indices. The results of these two Chi-square analyses and the presence of "disease" biovariants in both healthy and diseased animals of both species are not consistent with an increased risk of pasteurellosis at the interface, although his may be due to study design and methodological limitations,.

It is possible that there are uncommon biovariants that were not identified in these studies that can be responsible for outbreaks of disease. However, it is more likely that such biovariants would be identified in the retrospective studies (Chapters 3 and 4) or an outbreak in Hell's Canyon (Rudolph *et al.*, 2007). The isolation of Pasteurellaceae from both healthy and diseased animals is consistent with domestic animal models of pasteurellosis as an endogenous, opportunistic infection (Yates, 1982), or as incidental isolates. Data that permit estimation of measures of risk are required to more fully assess the potential for specific biovariants to be associated with disease in these species (Dohoo *et al.*, 2003).

Consistency of Pasteurellaceae results

The diversity of Pasteurellaceae that is apparent in this study, as well as the inconsistencies in biovariants that were identified between this study and retrospective studies (Chapters 3 and 4), are sources of variation that warrant further consideration.

The biovariant classification system was developed due to bighorn sheep Pasteurellaceae isolates that could not be classified with conventional serotyping (Jaworski *et al.*, 1998). The biovariant scheme assumes that an isolate's in vitro culture characteristics are consistent with its biological characteristics while inhabiting hosts. This assumption is difficult to test, and horizontal gene flow that might affect isolate pathogenicity may not be consistent with biovariant classifications (Kelley *et al.*, 2007). However, in vitro culture results have been useful for many microbiological studies and the biovariant system is presumed to provide the potential to distinguish among a number of Pasteurellaceae lineages. This fine-scale resolution is presumed to be superior to broader classification schemes when conducting studies concerned with transmission or disease due to Pasteurellaceae.

Ruminant oropharyngeal Pasteurellaceae appear to be best documented with tissue samples from tonsillar biopsies or tonsillar swab samples (Dunbar *et al.*, 1990) (Wild & Miller, 1991). As with previous studies (Wild & Miller, 1991), there was similarity in the isolates from antemortem oral swabs and postmortem tonsillar tissue for the bighorn sheep female that was euthanized due to injuries. In addition, there was an absence of isolates from pulmonary tissue, as might be expected in an otherwise healthy animal, based on domestic livestock models of pasteurellosis (Ackermann & Brogden, 2000). Although this supports the validity of the methods used, the results from this single animal are not definitive.

Temporal dynamics appeared to be a substantial source of variation in the Pasteurellaceae biovariants of domestic sheep and goats in this study. This phenomenon may extend to bighorn sheep. Identical biovariants were only isolated twice from the same individual for 22% of domestic sheep and 7% of domestic goats for populations

resampled six months apart. When it is considered that multiple isolates from the same individual were common, domestic sheep (5%) and domestic goat (4%) isolation events are more representative of how uncommon the same biovariant was recovered twice from the same individual. Similar observations were made when captive bighorn sheep were sampled twice for Pasteurellaceae (Weiser *et al.*, 2009). However, this latter example is not directly comparable to this study, due to administration of antibiotics in between bighorn sheep sampling events. Nevertheless, the unstated assumption of research to date is that a single oropharyngeal sample is representative of an animal's Pasteurellaceae microflora. This assumption may fail to account for the temporal dynamics of these biovariants. These temporal dynamics present challenges for establishing baseline values that could be used to identify pathogenic Pasteurellaceae.

Summary

This study primarily considered shared Pasteurellaceae among the host species studied, although there is interest in whether there is interspecies transmission of pathogenic agents (United States Geologic Survey/Bureau of Reclamation Office, 2006). It would not be surprising if some transmission of Pasteurellaceae occurred at the wildlife-livestock interface, based on previous reports of interspecific interactions (Foreyt & Jessup, 1982; Rudolph *et al.*, 2003; George *et al.*, 2008a), inferential data from this study, and the general potential for infectious agents to be introduced into naïve populations when there is interpopulation contact (Brauer & van den Driessche, 2001). However, much of the available information is anecdotal. Similarly, for our study, there are design limitations. If the data in this study is interpreted as evidence in favor of transmission, it implies that transmission is infrequent. Furthermore, the presence of

biovariants in apparently healthy and clinically diseased animals suggests that Pasteurellaceae are not consistently associated with disease.

Mycoplasma spp.

Mycoplasma was isolated from all but one domestic livestock population, but was not isolated from bighorn sheep. *Mycoplasma* has been associated with respiratory disease in domestic ruminants and free-ranging bighorn sheep (Parham *et al.*, 2006; Shiferaw *et al.*, 2006; Besser *et al.*, 2008; Rudolph *et al.*, 2007). Although the domestic livestock in this study were largely without clinical signs of disease, it is possible that subclinical infections were compromising productivity or will predispose animals to disease from other agents (Ruffin, 2001; Pugh, 2002). The absence of isolates from bighorn sheep suggests that these populations may be vulnerable to disease if this agent is introduced, or that this species is resistant to mycoplasmosis. The isolation of *Mycoplasma* during an outbreak indicates that infections and disease are possible in bighorn sheep (Besser *et al.*, 2008; Rudolph *et al.*, 2007). In contrast, *Mycoplasma* was not isolated from the euthanized bighorn sheep male that was associating with domestic sheep and goat populations where *Mycoplasma* was present. Therefore, further research is needed to clarify the impact of *Mycoplasma* on bighorn sheep and domestic ruminants.

Virology

The viral respiratory agents in this study were selected on the basis of their potential to cause respiratory disease or predispose to pneumonic pasteurellosis in domestic and wild ruminants (Ackermann & Brogden, 2000; Brogden *et al.*, 1998; Pugh, 2002; Van Campen *et al.*, 2001; Aune *et al.*, 1998). A high percentage of the domestic

livestock and bighorn sheep in this study had evidence of antibodies to PI-3 and BRSV (Table 6.8), and there was evidence for seroconversion for BRSV and PI-3 among domestic sheep and goats that were sampled twice. Parainfluenza-3 and BRSV (or reported as RSV) have been associated with respiratory disease in bighorn sheep, and domestic sheep and goats (Brako *et al.*, 1984; Brogden *et al.*, 1998; Yang *et al.*, 2008; Parks *et al.*, 1972; Spraker *et al.*, 1986; Rudolph *et al.*, 2007). However, evidence of antibodies in apparently healthy animals in these references and others (Parks & England, 1974; Spraker *et al.*, 1986; Clark *et al.*, 1985; Aune *et al.*, 1998; Schwantje, 1986; Rudolph *et al.*, 2007) indicate that survival from infections is possible and perhaps probable in populations with high serologic prevalences.

In contrast to PI-3 and BRSV, there were few animals with evidence of antibodies to BVD-1, BVD-2, and IBR. These viruses can be responsible for a range of respiratory and other clinical signs (Obando *et al.*, 1999; Pugh, 2002). There is limited documentation of the clinical effect of these infections in domestic sheep and goats (Zaghawa, 1998; Taylor *et al.*, 1977; Brako *et al.*, 1984; Yang *et al.*, 2008). There is serologic evidence of BVD and IBR infections in healthy bighorn sheep (Clark *et al.*, 1985). However, isolation of IBR from 3 of 6 lung samples from bighorn sheep during a Tendoys, Montana outbreak, isolation of BVD from 14 of 19 bighorn sheep lungs during a Lost Creek, Montana outbreak (Aune *et al.*, 1998), and > fourfold increases in serologic titers to BVD during the Hells Canyon outbreak (Rudolph *et al.*, 2007) suggest a role for these viruses in some die-offs.

When the results of previous studies are considered with this study, it appears that the viruses that were tested for in this study can be associated with disease in domestic livestock and bighorn sheep under some circumstances, but be apathogenic or mildly

pathogenic in others. Based on domestic ruminant models, these viruses may cause primary infections that result in secondary, opportunistic pneumonic pasteurellosis (Ackermann & Brogden, 2000; Brogden *et al.*, 1998). This model may apply to free-ranging bighorn sheep (Rudolph *et al.*, 2007). Populations that are naïve to these viral respiratory agents might be most vulnerable to outbreaks of respiratory disease if these agents are introduced. However, further research is needed to clarify the degree and circumstances under which these agents pose a risk for disease.

Cluster analysis

Cluster analysis was used as a strategy for determining whether there was segregation of Pasteurellaceae and viral serology results based on species and location relative to the interface. This method grouped individual animals' results based on similarities of binary values (present/absent) for each Pasteurellaceae biovariant and virus. An underlying assumption is that each agent is transmitted independently, although there is no data to support or refute this assumption. This analysis also assumed that the locations (interface or not) reflect true biological distinctions. The analysis resulted in highly significant differences among the four assigned clusters ($P < 0.0001$). However, these clusters did not correspond with the species-location designations (Table 6.9). Several reasons for this absence of correspondence are possible, including imprecision in the definition of interface locations, as this is an approximation for contact. In addition, temporal variation may obscure biological patterns that may exist. It is also possible that historic introductions of agents into naïve populations over the past century, across the wildlife/domestic interface, resulted in the maintenance of novel agents in new species, thereby obscuring previous distinctions in agent distribution. The latter explanation is

analogous to mosaic distributions of parasites as the consequence of host switching (Hoberg & Brooks, 2008). Regardless, of the explanation, distinct, species and location-based agent assemblages are not apparent from the data in this study.

Parasitology

Nine different genera or groups of nematode and coccidian genera were identified in fecal samples from study animals. These data are presented because of the potential for parasites to cause primary disease or to predispose animals to disease due to other agents (Thorne *et al.*, 1982; Pugh, 2002). As validated, standardized, quantitative methods for assessing parasite numbers were not available, only presence-absence data is reported for this study (Table 6.11?). These parasites are similar to those previously reported for domestic and bighorn sheep (Thorne *et al.*, 1982; Georgi, 1985). As *Muelleris* spp. is more commonly associated with domestic sheep than bighorn sheep (Pybus & Shave, 1984; Goldstein *et al.*, 2005), it is possible that the evidence for *Muelleris* spp. in noninterface bighorn sheep represents recent or historic introductions of this parasite to bighorn sheep. If true, this may support historic, interspecies introductions of bacterial and viral agents. This might explain the absence of evidence for species-location assemblages of bacterial and viral agents in the cluster analysis.

Conclusions

Pasteurellosis has long been a concern for outbreaks of respiratory disease in bighorn sheep, and is also responsible for sporadic outbreaks in domestic livestock. Domestic animal models of pasteurellosis indicate that the Pasteurellaceae are opportunistic pathogens that colonize the lower respiratory tract and cause disease when

there are adverse combinations of infectious agents, host characteristics, and environmental stressors (Yates, 1982; Czuprynski *et al.*, 2004; Zecchinon *et al.*, 2005; Dabo *et al.*, 2008). The isolation of many Pasteurellaceae biovariants from apparently healthy and clinically diseased animals in this study is consistent with this model, as is evidence from other studies that different agents may contribute to outbreaks under different circumstances (Aune *et al.*, 1998; Rudolph *et al.*, 2007). Consequently, pasteurellosis in domestic and bighorn sheep may be similar. As most biovariants were found in multiple species, further work is needed to clarify whether some biovariants are more likely to be associated with disease than would be expected by their prevalence in healthy animals.

This study did not rule out as yet unidentified agents, or rare or unique genetic recombinants of Pasteurellaceae as causes of outbreaks. However, the naiveté of some populations to *Mycoplasma* spp., BVD-1, BVD-2, or IBR suggests the potential for these agents to contribute to outbreaks.

Given the polarized nature of the debate over management practices at the bighorn/domestic sheep interface, there is the potential for the results of this study to be selectively interpreted. It will be more useful to reflect upon basic animal disease control principles and how they might be applied to free-ranging wildlife. It must be recognized that any time there is contact between different populations, there is potential for novel agents to be introduced to naïve animals. This concept has led to quarantine, vaccination, testing, risk assessment, and other strategies that are routinely applied to minimize spread of infectious disease among domestic animals, and to a lesser extent, humans (Zepeda *et al.*, 2001; Budd *et al.*, 2009).

Conventional disease control strategies minimize, but do not eliminate, the risk of introducing novel agents into populations. These strategies are applied with variable degrees of knowledge regarding the risks and consequences of different management options for specific pathogens. The level of knowledge for applying such principles to wildlife disease management is more limited. Consequently, for small or otherwise highly valued bighorn sheep populations, risk adverse strategies may be adopted, where all possible sources of agent introduction, competition for forage and space, and other risk factors may be considered as legitimate management options, even where the risk and benefits of these options is uncertain. Similarly, domestic sheep operations that are considered critical for a local economy, for exotic weed control, to prevent conversion of land to uses that are not compatible with wildlife or agricultural interests, or for other reasons, may require management strategies that protect their interests. For all other situations, management will be guided by sociological values and biological perceptions until the biological risks and options are clarified and a sociologically-based structure for decision making is agreed upon. There should be sufficient flexibility in such management policies so that unintended consequences can be recognized and addressed. Ideally, the results of this study will lead to identification of approaches that will be most useful for addressing biologically-based conflict at the bighorn/domestic livestock interface.

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Table 6.1. Characteristics of bighorn sheep and domestic sheep populations studied based on proximity to the bighorn/domestic sheep interface.

		Bighorn sheep[1]		Goats[2]	Domestic sheep[2]	
		Non-interface populations[3]	Interface populations[3]	Interface populations[3]	Interface populations[3]	Non-interface populations[3]
No. populations		7	3	1	6	6
Population size	Mean ± S.D.	274.1 ± 247.4	198.1 ± 141.5	925[4]	467.1 ± 656.7[4]	1030 ± 1509.1[4]
	Range	35 - 750	70 – 350	-	25 – 1780	30 – 4000
Population density (No./km)	Range	0.3 – 1.9	0.292 – 0.703	-	-	-
No. animals sampled	Total	234	106	45	152	219
	Range per population	6 – 81	26 - 49	-	19 – 70	20 - 70
Land occupied - winter	Public	7	3[5]	0	0	2
	Private	0	[5]	1	6	4
Land occupied - summer	Public	7	3[5]	[6]	[6]	3
	Private	0	[5]	1[6]	6[6]	3

[1]Data based on 2003 annual aerial census by Montana Fish, Wildlife and Parks
[2]Data from questionnaire from this study
[3]Based on 14.5 km barrier recommended for land management (United States Department of the Interior, 1998); interface ≤ 14.5 km, relative to sympatric species, and non-interface > 14.5 km, relative to sympatric species (or surrounded by development that prevents interactions with sympatric species)
[4]Number of females in population
[5]One population 50% federal and 50% private land
[6]One population 10% on public land

Table 6.2: Interface and non-interface bacterial isolates from bighorn sheep (n = 10 populations), domestic sheep (n = 12 populations), and goat (n = 1 population) sampled prospectively, in comparison with retrospective studies of bighorn sheep and domestic sheep.

	Host species		Bighorn sheep		Goat	Domesticsheep		Bighorn(Retro.)[1]		Domestic(Retro.)[2]	
	Health[3]		Healthy	Healthy	Healthy	Healthy[4]	Healthy[5]	Diseased	Healthy	Diseased	Healthy
	Interface[6]		No	Yes	Yes	Yes	No	-	-	-	-
	No. populations		7	3	1	6	6	-	-	-	-
	No. animals		234	106	45	152	219	-	-	-	-
	No. isolates		506	294	355	873	912	104	663	734	144
Species[7]	Type	Excptn[8]									
Actinobacillus	n/a	n/a	2		12	62	30			2	1
Coliform	n/a	n/a	3	5	15	42	21			1	
Mannhemia	1	α	6	3	6	7	12		11	9	1
haemolytica		αB			2	2	2		8	4	
		αBG				2			1	1	
		αg			1	1	1			3	
		B			1					1	
		E			9		2		3	2	
		EG			1	1	1			29	5
		G		4	10	22	16			172	8
		n/a	4	4	68	80	102	4[9]	14	9	1
	10	α	9			9	21	2	8	9	1
		αBE	1	1					1		
		αC				1			1		
		B		2					3		
		C			1		1				1
		n/a	5			12	36		5	4	1
	11	α				13	3		2	2	1
		αE				1					1
		n/a	6			66	66		5	50	3
	16	α	3				6		2	3	1
		αB					2		4	3	
		αBE				3	5			12	
		αE			2	16	9	1		5	7
		αEG				2				10	
		B					5		1		
		BE				1	3			2	
		E					8			11	2
		EG					1			9	
		G					1			2	
	3	α				1	1		7		
		αCD		1					1		
		αG				1			1		
		B	2						1		1
		CDE		2					3		
		n/a			8	64	68	10[9]	9	18	7
	5	α				4				1	2
		αB				4	2		1		1
		B					3				2
		n/a				23	30		18	15	3
	6	αr	1	1			2			2	

	Host species		Bighorn sheep		Goat	Domesticsheep		Bighorn(Retro.)[1]		Domestic(Retro.)[2]	
	Health[3]		Healthy	Healthy	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy
	Interface[6]		No	Yes	Yes	Yes	No	-	-	-	-
Species[7]	Type	Excptn[8]									
Mannhemia haemolytica	7	B				3	15		1	5	2
		BX				3	7		1	16	2
		X			4	10	6			27	4
		n/a			8	7	12	4[9]	1	6	3
	8	B		2		11	6			6	2
		n/a	3	3	18	16	36		5	2	7
	9	αβB	1	1			1		1		
		αβR		3					9		
	U	α	1				2		2		
		αB		1					2		
		αβB			1		4			13	
		αβBX					4			3	
		αβ		1	7	1	26		7	1	7
		αβX				3	3			1	
		βBE				1				6	
		βBEX					8	1[9]	3		
		βB		1		1			1	5	
		βBX				4	2			8	1
		β			48		8	3[10]	2	6	9
Pasteurella multocida	A	n/a	3	12	6	21		1[9]	2	10	
	B	n/a				21	3	1[9]	6	6	
	canis	n/a				4				4	
	septi	n/a	4	6	10	35	10		2		
	U16	n/a			2				1		1
	U 23	n/a					1		1		
	U6	n/a		6	9	9		2[9]	2	7	
Pasteurella (Bibersteinia) trehalosi	11	e		12	2	20	16			10	1
	2	B	140	105		3		14	130	1	
		BE	11	11					1		
		BG		4				1			
		BS		13					13		
		C	1	1	8	2	11		4	2	
		CD	1		2				1	6	
		CDES				1	1			1	
		CDS			2	2	2	2[9]		4	
		CS				2			1		
		E	4	6		10	4		6	5	1
		GS	1			1			1		
		S		1		2	1			1	
		n/a	152	64	84	208	228	42[11]	204	115	23

	Host species		Bighorn sheep		Goat	Domesticsheep		Bighorn(Retro.)[1]		Domestic(Retro.)[2]	
	Health[3]		Healthy	Healthy	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy
	Interface[6]		No	Yes	Yes	Yes	No	-	-	-	-
Species[7]	Type	Excptn[8]									
Pasteurella	4	B	4					1	6		
(Bibersteinia)		BCDS					1			3	
trehalosi		CD					1			1	
		CDE					3		1		
		CDES				2	6			2	1
		CDS			8	28	6		4	3	2
		n/a					6	5[12]	5	1	4
Not Identified	n/a	n/a	138	18		2	12		8	11	

[1]Retrospective study – Chapter 3
[2]Retrospective study –Chapter 4
[3]Healthy = no signs of respiratory disease; diseased = signs of upper or lower respiratory disease
[4]142 domestic sheep without signs of respiratory disease, 10 domestic sheep with signs of mild respiratory disease
[5]218 domestic sheep without signs of respiratory disease, 1 domestic sheep with signs of mild respiratory disease
[6]Yes ≤ 14.5 km to sympatric species; No > 14.5 km to sympatric species or surrounded by development that prevents interspecific interactions
[7]Bacterial isolate species
[8]Exceptions
[9]All juveniles
[10]Two juveniles
[11]29 juveniles
[12]Four juveniles

Table 6.3. Pasteurellaceae biovariants that were not identified in both this study and retrospective studies (Chapters 3 and 4) of bighorn and domestic sheep.

Biovariants identified in bighorn sheep in a retrospective study (Chapter 3) but not in bighorn sheep in this study			Biovariants identified in bighorn sheep this study but not in bighorn sheep in a retrospective study (Chapter 3)			Biovariants identified in domestic sheep a retrospective study (Chapter 4) but not in domestic sheep in this study			Biovariants identified in domestic sheep in this study but not in domestic sheep in a retrospective study (Chapter 4)		
Biovariant			Biovariant			Biovariant			Biovariant		
Species	Type	Expn.	Species	Type	Expn.	Species	Type	Expn.	Species	Type	Expn.
H.somnus	n/a	n/a	*Actinobacillus*	n/a	n/a	*Campylobacter*	n/a	n/a	Mhem	3	αG
Mhem	1	αE	Coliform	n/a	n/a	Mhem	10	αG		3	α
		αB	Mhem	1	G			αBE		16	B
		αBS		6	αR		11	αβ		10	αC
	10	αE		8	B			B		16	B
		αβ	Ptre	11	E			BE		9	αβB
		BES						αβBE		U	α
		βB						αG		U	βBEX
		E					16	βBE	Pmult	septi	n/a
	11	αβG						n/a		U23	n/a
		αGX					2	S	Ptre	2	CS
		αβ					3	ABCDE		2	GS
	2	S						αBC		4	CDE
	3	αB						αBCD			
		αBE					5	αβ			
		αBEX						BCD			
		αC						BD			
		αE						CDS			
		αES						E			
		BCX						βB			
		BE					6	α			
		BEX						αB			
		BX						R			
		E						RX			
	5	β					7	BG			
	6	α						βBX			
		R						G			
		RX					8	βB			
		n/a					9	αβ			
	7	B						B			
		BX						β			
	8	β					U	αBE			
	9	αBR						αBER			
		αBRX						αβBG			
		αβ						αER			
		αR						E			
		B						βE			
		βR						βBEX			
	U	αβBC						βE			
		αβBERX						βX			
		αβE									

Biovariants identified in bighorn sheep in a retrospective study (Chapter 3) but not in bighorn sheep in this study			Biovariants identified in bighorn sheep this study but not in bighorn sheep in a retrospective study (Chapter 3)			Biovariants identified in domestic sheep a retrospective study (Chapter 4) but not in domestic sheep in this study			Biovariants identified in domestic sheep in this study but not in domestic sheep in a retrospective study (Chapter 4)		
Biovariant			Biovariant			Biovariant			Biovariant		
Species	Type	Expn.	Species	Type	Expn.	Species	Type	Expn.	Species	Type	Expn.
Mhem	U	αER				Pmult	galli	n/a			
		αR					stomat	n/a			
		βBX					testu	n/a			
		αβB					u12	n/a			
		n/a					u18	n/a			
Pmult	galli	n/a					u20	n/a			
	testu	n/a					u26	n/a			
	u11	n/a					n/a	n/a			
	u8	n/a				Ptre	2	αB			
	U2	n/a						D			
Ptre	2	αB					4	CS			
	4	EDG						S			
		βBS									
		BS									
		DGS									
		DS									
		S									

Expn = Exception
H. somnus = *Haemophilus somnus*
Mhem = *Mannheimia haemolytica*
Ptre = *Pasteurella* (*Bibersteinia*) *trehalosi*
Pmult = *Pasteurella multocida*
septi = *septicemia*
galli = *gallisepticum*
stomat = *stomatus*
testu = *testudin*

Table 6.4. Bacterial isolates from oral swab and tonsillar tissue from a bighorn sheep female euthanized due to capture related injuries.

Bacteria	swab	tonsil
Bacillus spp.	X	
Pasteurella (*Bibersteinia*) *trehalosi* 2[be]	X	X
Pasteurella (*Bibersteinia*) *trehalosi* 2[b]	X	X
Streptococcus spp.	X	X

Table 6.5. Bacterial isolates from oral swab and lung tissue of a bighorn sheep male that was euthanized after co-habitating in shelters containing domestic sheep and domestic goats.

	Euthanized bighorn sheep male sample		Isolates from nearest bighorn sheep population	Isolates from co-habitating domestic sheep	Isolates from co-habitating domestic goat
Sample type	Swab	Lung	Swab	Swab	Swab
P. (B.) trehalosi 2^{CDS} *	X	-	-	-	X
P. (B.) trehalosi 2^{B} *	X	X	X	X	-
P. (B.) trehalosi 2 *	X	X	X	X	X
P. (B.) trehalosi 4^{CDS} *	X	-	-	X	X
Bacillus spp		X	X	X	X
Arcanobacterium pyogenes		X	X	X	X

* *P. (B.) trehalosi* = *Pasteurella* (*Bibersteinia*) *trehalosi*

Table 6.6. Pasteurellaceae results from individual domestic sheep and domestic goats sampled twice, six months apart.

	Domestic sheep	Domestic goat
No. populations	3	1
No. individuals	85	34
No. of unique isolate events[1]	493	219
No. of individuals with one isolate identified at both sample events	20	9
No. of individuals with two isolates identified at both sample events	2	0

[1]Each biovariant isolated from an individual was considered an isolate event. The sum of isolates for each individual at both sample events, less those identified twice in the same individual, was used as a measure of instances where a biovariant could be isolated ≥ 1occasion in the same individual.

Table 6.7. *Mycoplasma* spp. isolates from bighorn sheep, domestic sheep, and domestic goats in interface and non-interface populations.

	Bighorn sheep		Domestic goat	Domestic sheep	
Location	Non-interface	Interface	Interface	Interface	Non-Interface
Number of populations	6	3	1	6	6
Number of populations with *Mycoplasma* spp. isolates	0	0	1	5	6
Number of individuals tested for *Mycoplasma* spp.	133	101	14	56	110
Percentage of individuals tested with isolates of *Mycoplasma* spp.	0%	0%	22%	72% (SD ± 9.6%)	66% (SD ± 14.7%)
Number of individuals with evidence of respiratory disease tested for *Mycoplasma* spp.	0	0	0	4	0
Number of individuals with respiratory disease and *Mycoplasma* spp. isolates	0	0	0	1	0

Table 6.8: Number (%) of bighorn sheep, domestic sheep, and domestic goats with serologic evidence for antibodies to parainfluenza -3, bovine respiratory syncytial virus, bovine viral diarrhea-1 and 2, and infectious bovine rhinotracheitis in interface and non-interface populations.

	Bighorn		Goat	Domestic sheep	
	Non- interface	Interface	Interface	Interface	Non-Interface
Number of populations	7	3	1	6	6
Number of animals tested	198	105	44	143	214
Parainfluenza -3	165 (83%)	91 (87%)	9 (21%)	102 (71%)	113 (53%)
Bovine respiratory syncytial virus	57 (29%)	76 (72%)	44 (100%)	95 (66%)	104 (49%)
Bovine viral diarrhea-1	0	0	0	1 (0.7%)	3 (1%)
Bovine viral diarrhea-2	0	0	0	1 (0.7%)	6 (3%)
Infectious bovine rhinotracheitis	0	2 (2%)	1 (2%)	0	0

Table 6.9. Summary of cluster assignments for individual bighorn sheep and domestic sheep and goats based on species-location characteristics ($P < 0.0001$).

Variables		Cluster 1	Cluster 2	Cluster 3	Cluster 4
Bighorn - non-interface	No. (Row %)	11(5)	139(61)	70(31)	8(3)
Bighorn – interface	No. (Row %)	20(19)	39(37)	46(44)	0 (0)
Domestic - interface	No. (Row %)	39(23)	48(29)	41(25)	38(23)
Domestic – non-interface	No. (Row %)	9(5)	87(47)	62(33)	29(16)
	Mean(Row%)	19.8(13.0)	78.3(43.5)	54.8(33.3)	18.8(10.5)

Table 6.10. Parasites identified in bighorn sheep, domestic sheep, and domestic goats in populations near to and distant from the wildlife/domestic livestock interface.

	Bighorn sheep		Domestic goat	Domestic sheep	
	Noninterface	Interface	Interface	Interface	Noninterface
Number of populations Evaluated	6	3	1	6	3
Number of animals evaluated	165	98	12	44	36
Parasite species (No. of host populations present)	*Protostrongylus* spp.	*Protostrongylus* spp.	*Eimeria* spp.	*Eimeria* spp.	*Eimeria* spp.
	Muelleris spp.	*Dictyocaulus* spp.	*Cooperia* spp. – *Trichostrongylus* spp. - *Ostertagia* spp.[1]	*Cooperia* spp. - *Trichostrongylus* spp. - *Ostertagia* spp.[1]	*Cooperia* spp. - *Trichostrongylus* spp. - *Ostertagia* spp.[1]
	Dictyocaulus spp.		*Nematodirus* spp.	*Haemonchus* spp.	*Haemonchus* spp.
			Moniezia spp.	*Nematodirus* spp.	*Nematodirus* spp.
			Strongyloides spp.	*Moniezia* spp.	*Moniezia* spp.
				Dictycaulus spp.	
				Strongyloides spp.	

[1] *Cooperia* spp., *Trichostrongylus* spp., and *Ostertagia* spp. were not differentiated

CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

This study was conducted to gain additional information about the potential causes of respiratory disease outbreaks in bighorn sheep. Because domestic sheep have been hypothesized to be a reservoir of Pasteurellaceae that are the primary cause of such outbreaks (Council for Agricultural Science and Technology (CAST), 2008), this dissertation was primarily focused on Pasteurellaceae biovariants responsible for respiratory disease, and domestic sheep as potential reservoirs. However, due to reports suggesting that other agents could be involved in respiratory disease outbreaks, this study also included research on *Mycoplasma*, viral agents, and endoparasites that could be determinants of respiratory disease in bighorn sheep (Pillmore, 1961; Aune *et al.*, 1998; Rudolph *et al.*, 2007; Besser *et al.*, 2008).

Observations of bighorn sheep respiratory disease outbreaks have lead to assumptions that a transmissible infectious agent is responsible. If this is true, it is important to identify the reservoir for this agent as a means of developing control strategies. As it was not possible to conduct a study that fully addresses these questions, this dissertation presents baseline data for preliminary assessments of agents that could be responsible for respiratory disease in bighorn sheep. This is needed to provide perspective on agents isolated from animals with respiratory disease during outbreaks. This baseline data also provides a foundation for subsequent studies on the magnitude of effect and frequency of occurrence of outbreaks due to specific agents.

The design of this study incorporated several concepts that were considered important for advancing knowledge on the causes of respiratory disease outbreaks. These concepts included:

- Data from both bighorn and domestic sheep - Limited data has been published on the Pasteurellaceae of healthy bighorn and domestic sheep using the biovariant classification scheme (Ward *et al.*, 1997; Jaworski *et al.*, 1998; Tomassini *et al.*, 2009). This dissertation provides data on the biovariants of sympatric bighorn and domestic sheep for the purpose of identifying biovariants that are shared, and those that are associated with a single species. Such information could help with identification of reservoirs, if there is interspecies transmission of agents in either direction.
- Multiple populations of healthy animals – Studies of outbreaks are generally limited to case reports of animals with clinical disease. Such studies have limited inference, in comparison with studies of multiple populations. Consequently, this dissertation provides a more comprehensive assessment of the Pasteurellaceae of bighorn and domestic sheep than is possible from case reports. This facilitates the interpretation of data from animals with disease (the numerator) by providing a more rigorous assessment of baseline Pasteurellaceae in animals without disease (the denominator).
- Data from populations near to and distant from the bighorn/domestic sheep interface – Sampling of bighorn and domestic sheep populations that were in proximity provided an opportunity to describe the agents shared by populations at the same interface. Populations distant from the interface provide perspective for understanding whether interface populations have characteristics which differ from populations where inter-specific agent transmission is not possible. This

dissertation presents data that permits a degree of inter- and intra-specific comparisons that have not previously been possible.

- Sampling for multiple agents – Data from Montana bighorn sheep outbreaks identified multiple agents in animals with respiratory disease (Aune *et al.*, 1998). This is consistent with multiple causative agents of respiratory disease in other species (Blood & Radostits, 1989; Pugh, 2002). Consequently, this dissertation presents data on multiple agents that were concurrently tested for. This is consistent with scientific approaches that pursue multiple hypotheses as a rigorous means of identifying the best hypotheses for describing natural phenomena (Chamberlin, 1965).
- Resampling of individuals – An unstated assumption of previous work has been that a single oropharyngeal sample of an individual can provide data that is representative of the animal's Pasteurellaceae microflora. If this assumption is not valid, it affects the interpretation of single sample events and suggests that alternative sampling strategies should be considered. This dissertation presents Pasteurellacea data from individuals in three domestic sheep and one domestic goat population that were resampled six months apart.

As an outbreak did not occur in the populations that we studied during the course of our investigation, retrospective data was used to provisionally identify biovariants that could be associated with respiratory disease in bighorn and domestic sheep. This permitted provisional comparisons of the Pasteurellaceae microflora of animals with respiratory disease and those that were apparently healthy.

The important outcomes of this dissertation and their implications were:

- There were many (> 200) Pasteurellaceae biovariants identified in the animals in this study, most of which had a prevalence of < 7%.
    - Implication: Research comparing the pathogenicity of Pasteurellaceae biovariants will require datasets larger than were possible for this study to address most questions, as smaller datasets may have insufficient power to detect differences that exist (Type II error).
- Pasteurellaceae biovariants were generally found in both apparently healthy animals and those with respiratory disease.
    - Implication: Additional data from apparently healthy animals and those with respiratory disease are needed to estimate the odds of a given biovariant being associated with respiratory disease. Biovariants with the highest odds of being associated with disease may be worthy targets for subsequent research to establish their pathogenicity. However, the magnitude and frequency of outbreaks due to a given biovariant may be more important for identifying biovariants that are of significant concern.
- Although some Pasteurellaceae biovariants appeared to be primarily associated with a single species, most were found in multiple species.
    - Implications: Additional data is needed to determine whether one species can serve as a reservoir of pathogenic Pasteurellaceae for sympatric species.
- There was substantial temporal variation in the Pasteurellaceae of the individuals that were resampled.

- Implications: There is a need to identify the optimal means of sampling the species in this study so that the Pasteurellaceae microflora is adequately characterized.

- Bighorn sheep were naïve to *Mycoplasma* spp, and each species studied were largely naïve to BVD and IBR
    - Implications: When considered in combination with other publications that suggest a role for these agents in the development of respiratory disease (Taylor *et al.*, 1977; Aune *et al.*, 1998; McAuliffe *et al.*, 2003; Besser *et al.*, 2008), there is a need to clarify the degree (magnitude and frequency) to which these agents contribute to respiratory disease in the species studied.

The results of these studies are more consistent with models of multiple pathogens as causes of respiratory disease, than of single, primary infectious agents. This suggests that a complex of determinants could be responsible for respiratory disease in these species. Consequently, there is a need to clarify the determinants of respiratory disease, the degree to which each determinant is responsible for disease, and the potential for reducing the magnitude and frequency of respiratory disease outbreaks by managing the determinants most responsible for respiratory disease.

Future Directions

Much remains to be determined for understanding respiratory disease outbreaks in bighorn sheep. Further investigation is needed to clarify the role of Pasteurellaceae in bighorn sheep respiratory disease. In addition, as the data in this dissertation suggests that

multiple determinants may be responsible for respiratory disease in bighorn sheep, it would be prudent to pursue additional agents and determinants of respiratory disease. Some of the research projects that could be conducted to address the relevant questions include the following:

- Pasteurellaceae-related projects:
    - There is a need to determine the degree to which Pasteurellaceae biovariants vary temporally. If temporal variation is a common phenomenon, there is a need to determine optimal sampling strategies.
    - There is a need to determine the degree to which Pasteurellaceae biovariants vary spatially. If there is substantial geographic variation it may not be possible to pool data from different regions. If this is the case it will be difficult to make generalizations over a broad geographic range, and region-specific research may be required to identify agents responsible for causing respiratory disease.
    - Investigations are needed that will permit estimation of the odds of the association between individual Pasteurellaceae biovariants and animals with respiratory disease. This will require representative samples of apparently healthy animals and those with respiratory disease. This will also require labeling of samples with unique animal identification numbers. Depending on the results, this information might be complemented by studies where animals are inoculated with suspected pathogenic biovariants under controlled conditions.

- Longitudinal studies are needed to document transmission of Pasteurellaceae and other respiratory agents. This might occur under controlled conditions. However, conducting such studies on free-ranging populations will be more appropriate for assessing the actual risks of inter-specific transmission. Such studies may occur when domestic livestock are being used for exotic weed control, during seasonal grazing, or other settings.
- If there is documentation of transmission of Pasteurellaceae biovariants that can act as primary pathogens, there is a need to identify reservoirs of these biovariants.
- There is a need to consistently utilize laboratories that are experienced with the isolation of Pasteurellaceae and have a high success rate of isolating Pasteurellaceae when it is present. It is also important that such laboratories be capable of identifying biovariants, as well as further characterization of isolates by molecular methods when needed. Maintenance of an archived collection of isolates will maximize the research benefits of this work, as it may help to identify genotypic characteristics associated with pathogenicity. The Caine Veterinary Teaching Center is a laboratory that meets these requirements.
- There is a need to employ consistent protocols and methods for investigating disease outbreaks, as this is the only means by which results from outbreaks can be rigorously integrated or compared.

- Multiple agent investigations

- There is a need for future investigations that concurrently sample for *Mycoplasma* spp., viral agents, and parasites as potential causes of respiratory disease. There is a need to validate serologic assays for viral agents when applied to bighorn sheep, and to employ assays for parasites that have biological relevance. The previously listed studies that are needed for Pasteurellaceae are relevant to all agents that are potential respiratory pathogens, and this facilitates concurrently conducting investigations for multiple agents.

- Non-infectious agent determinants
  - Multiple determinants may contribute to respiratory disease outbreaks. It is important to identify important determinants that may be targets for management activity. It also is important to identify determinants that cannot be managed, but that must be considered when setting management objectives. Potential areas of research include:
    - Forage nutritional content
      - There is a need to develop methods of sampling range for macro and micronutrient content. This will be valuable for determining the carrying capacity of range for a single species, as well as consideration of the impact of sympatric grazing species. This will permit evaluation of whether outbreaks can be associated with populations that exceed carrying capacity or nutrient deficiencies.
    - Weather

- There is a need to identify weather-related factors that could influence outbreaks and population dynamics. These may be direct effects, such as adverse weather that directly affect animals, or indirect effects, such as those that influence forage quality and quantity.

- External stressors
  - There is evidence that external stressors can predispose bighorn sheep to disease outbreaks (Spraker *et al.*, 1984). However, this hypothesis has not been tested in free-ranging populations and is generally not considered in studies under controlled conditions.
- Population
  - There is a need to establish consistent and valid methods for sampling populations for recruitment, mortality, and demographic characteristics. There are substantial limitations to the methods available for directly estimating population size or indirectly evaluating population dynamics with indices, such as lamb-ewe ratios (Festa-Bianchet, 1992; Bodie *et al.*, 1995; McCarty & Miller, 1998; Rabe *et al.*, 2002). Quantitative measures or indices that are accurate and have biological relevance are needed for longitudinal studies of single populations and inter-population comparisons, as well as for outbreak

investigations. Although there is promise for the development of new methods (Bernatas & Nelson, 2004), future investigations will benefit from more accurate population data, as well as clear and consistent characterizations of outbreaks.

- Individuals
  - Some previous reports of bighorn sheep respiratory disease outbreaks have provided qualitative assessments of the body condition (body fat) of individual's that die. However, consistent and validated methods that permit intra and inter-population comparisons have not been employed. Similarly, validated methods for identifying animals with subclinical respiratory infections are needed. In addition, determination of micronutrient levels and similar measures of animal health may be useful for indentifying individuals with a greater risk of developing respiratory disease.
- Integrated indices
  - It is likely that integrating multiple indices of animal health or other determinants will be most useful for predicting populations that are at risk of respiratory disease outbreaks. Such integrated indices will also be useful for evaluating the efficacy of different management strategies for the prevention and amelioration of outbreaks. Such indices and

management strategies must be incorporated into regional management plans on a dynamic basis, based on existing conditions.

Research is needed to clarify the determinants of health and disease in bighorn sheep and sympatric ungulates. This research must be focused on identifying practical and valid means of identifying and managing determinants of respiratory disease in these populations. This will be valuable for reconciling some of the debate on land use policy for these species. However, the underlying core values of stakeholders frame much of the debate. Consequently, there is a need to address the sociological and communication issues that exist to resolve many of the sources of contention.

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