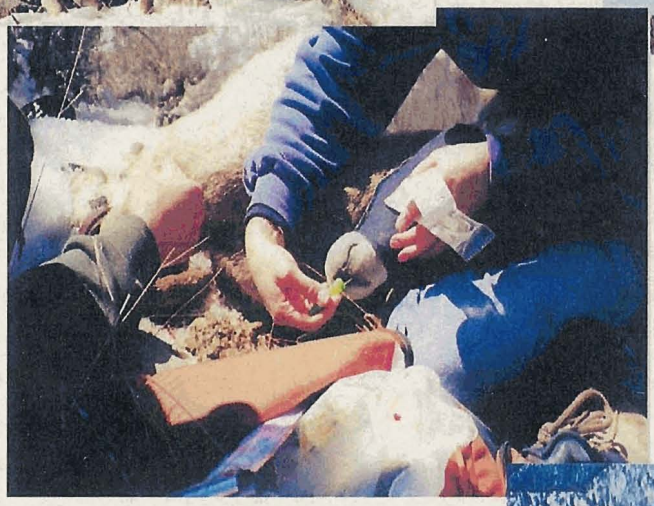
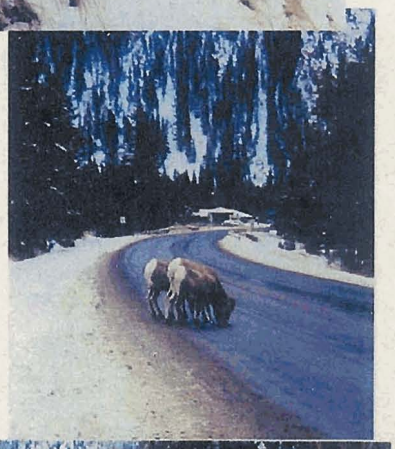
HERD HEALTH AND HABITAT QUALITY IN RELATIONSHIP TO *PASTEURELLA SPP.* INDUCED PNEUMONIA IN ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS)*



- Masters of Veterinary Science -

Rebecca K. Frey
August 2006

HERD HEALTH AND HABITAT QUALITY IN RELATIONSHIP TO *PASTEURELLA* SPP. INDUCED PNEUMONIA IN ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS*)

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By

Rebecca K. Frey

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Major Professor: Alton C. S. Ward, Ph.D.

AUTHORIZATION TO SUBMIT

THESIS

This thesis of Rebecca K. Frey, submitted for the degree of Master of Science with a major in Veterinary Science and titled "Herd health and habitat quality in relationship to *Pasteurella* spp. induced pneumonia in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*)," has been reviewed in final form, as indicated by the signatures and dates given below. Permission is now granted to submit final copies to the College of Graduate Studies for approval.

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DEDICATION

I dedicate this thesis to my family, friends and especially my husband who has been by my side since I began this project. I would also like to dedicate this thesis to all of my friends and supporters at Caine Veterinary Teaching and Research Center, in particular, Dr. Alton Ward and Dr. Glen Weiser.

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CHAPTER ONE

LITERATURE REVIEW

INTRODUCTION

Pasteurella/Mannheimia haemolytica and *Pasteurella trehalosi* are known as critical factors contributing to pneumonia epizootics in bighorn sheep (Marsh, 1938; Spraker and Hibler, 1982; Thorne et al., 1982, Ward et al. 1990; Dunbar et al. 1990). However, multiple factors predispose bighorn sheep to pneumonic pasteurellosis and may be just as important to consider in preventing a pneumonia die-off. These factors include viral infections, poor nutrition, high lungworm loads, other bacterial infections or any undue stresses such as population density, human disturbance, inter- and intra-specific species competition, predator harassment, adverse weather, and livestock competition (Spraker et al. 1984; Buechner, 1960, Forrester, 1971; McCollough et al. 1980; Wakelyn, 1987; Coggins, 1988).

Factors that differ between disease affected and non-affected populations have not been well characterized. The first recognized epizootic of bighorn pneumonia was recorded by Rush (1927) who described the Sun River herd of Montana as having declined by 75% with evidence of pneumonia detected at necropsy. Since then, numerous reports of pneumonia in bighorn sheep have been made with some populations being entirely decimated by the events (Potts, 1937; Marsh, 1938; Post, 1962; Wishart et al., 1980; Spraker et al., 1984; Onderka and Wishart, 1984; Bailey, 1986; Festa-Bianchett, 1988). However, information about the herds was collected after the die-offs had already occurred and the populations had suffered severe losses. While the information collected from these herds over the years is invaluable, knowledge of normal flora and potential pathogens carried in healthy bighorn populations may help wildlife

managers better understand how those populations should be managed in the future to prevent disease.

PASTEURELLOSIS IN BIGHORN SHEEP

Rush (1927) first reported pneumonia in bighorn sheep by describing a disease in the Sun River herd of Montana that included poor body condition, lungs adhered to the pleural cavity, abscesses within the lung tissue and outward coughing and malaise. These terms would come to describe nearly every bighorn pneumonia epizootic since and are consistent with a diagnosis of bacterial pneumonia. In 1937, Potts described another pneumonia epizootic in Rocky Mountain National Park, Colorado where *Pasteurella ovisepticus* (see also *Pasteurella multocida*) was isolated from the lungs of a dying bighorn ram. Marsh (1938) summarized four outbreaks of pneumonia in the Sun River herd, Yellowstone National Park, and Glacier National Park from 1925 to 1937. Similarities were found among the four die-offs including coughing, poor condition and bronchopneumonia, with lung consolidation and adhesions. Marsh described the bronchopneumonia histologically as an infiltration of monocytes and lymphocytes into the bronchial epithelium with an exudate of leucocytes on the bronchial mucosa as well as in the alveoli and bronchioles. *Protosyrongylus stilesi* larvae cysts were seen in the lungs of sheep and bacterial culture revealed *Corynebacterium pyogenes*; now identified as *Arcanobacterium pyogenes* (Reddy et al., 1982; Ramos et al., 1997); and *Pasteurella* spp. From this work the original diagnosis of bighorn pneumonia caused by lungworm infection followed by bacterial invasion was made. Since then, much more data has been gathered on the disease known as the bighorn pneumonia complex.

In Montana, outbreaks of respiratory disease have plagued bighorn sheep across the state. More recently pneumonic epizootics occurred in bighorn sheep in the Tendoy Mountains (1991), Lost Creek (1993), Highland-Pioneer (1994), and Quake Lake (1996)

herds which resulted in 75%, 55%, 87% and 60% mortality respectively (Aune et al. 1998).

In Colorado, several die-offs have occurred where bronchopneumonia was diagnosed. Many were attributed to high lungworm loads in the adults and subsequent transplacental transmission to the lambs (Spraker and Schmidt, 1983; Bailey, 1986). Affected lambs developed lungworm cysts after birth and often died within the first two months of life from "lamb pneumonia" (Spraker and Schmidt, 1983; Thorne et al., 1982). Die-offs occurred in the Tarryall Mtns. from 1921-24 where 338 of 350 (96%) bighorn sheep were reported to have died and in Rocky Mountain National Park in 1935 where hemorrhagic septicemia was first described in adult rams (Potts, 1937; Forrester and Senger, 1963). Die-offs also occurred in the Pikes Peak herd in 1930 and again in 1952 along with the Tarryall's again, and Kenosha ranges where populations decreased from 1500 to 500 bighorn sheep (Buechner, 1960). Descriptions of the disease included hemorrhagic septicemia, lungworm infection and verminous pneumonia.

In Wyoming, pneumonia outbreaks were reported as early as the 1920's when the Yellowstone National Park herd was reduced from 346 to 77 animals (Forrester and Senger, 1963). In Jackson Hole fifty percent of the 1200 bighorn sheep died in 1936-37 (Forrester and Senger, 1963). In the 1950's, the captive semi-wild herd at the Sybille Wildlife Research unit experienced an all age die-off. *Pasteurella* spp. were the predominant bacteria recovered from pneumonic lungs of sheep (Thorne et al., 1982).

Die-offs have been documented in British Colombia since 1940 when heavy mortality occurred in the Stoddart-Radium bighorn sheep population after a domestic sheep flock was introduced. In 1964, bronchopneumonia was diagnosed in the Bull

River population where only 8 of 250 bighorn sheep survived (DeMarchi and Davidson, 1983). In 1965, ewe carcasses were discovered in the Wigwam herd and the population decline was noted as 38-50% followed by low lamb survival (DeMarchi and Davidson, 1983; Schwantje, 1988). Other die-offs over the next three years included the Premier Ridge herd that suffered 72% mortality, the Columbia Lake herd with 73% mortality, and the Stoddart Creek herd which had over 75% mortality (Schwantje, 1988). Similar die-offs occurred in most of these herds as well as others (Wildhorse and Lussier ranges) in the early 1980's and respiratory disease was implicated in all of them (Schwantje, 1988). More recently, a population of California bighorn sheep was reduced by nearly 75% in the South Okanagan due to the rapid spread of respiratory disease (Ethier, 2000).

Similar mortalities were reported to have occurred in Alberta where nearly 300 sheep died at Waterton Park in 1982 (Wishart, 1983). Bacteria isolated from dead sheep were primarily *P. trehalosi* (Wishart, 1983). However, the first major die-off of sheep occurred in 1937 in Waterton, when 75% of the population died (Stelfox, 1971). Another die-off occurred in the Kootenay population in 1941 when 85% of that population died with what was diagnosed as verminous pneumonia (Stelfox, 1971). Stelfox listed numerous similar reports from the Vermillion range, Palliser range, Bow River, Livingstone-Highwood range and Jasper between 1942 and 1950. The Jasper bighorn sheep declined from nearly 2500 to 400 animals over three winters (Stelfox, 1971). Festa-Bianchet (1988) reported a smaller die-off in the Sheep River herd in 1986 with about 40% mortality. Only *P. haemolytica* was recovered from dead bighorn sheep in this epizootic.

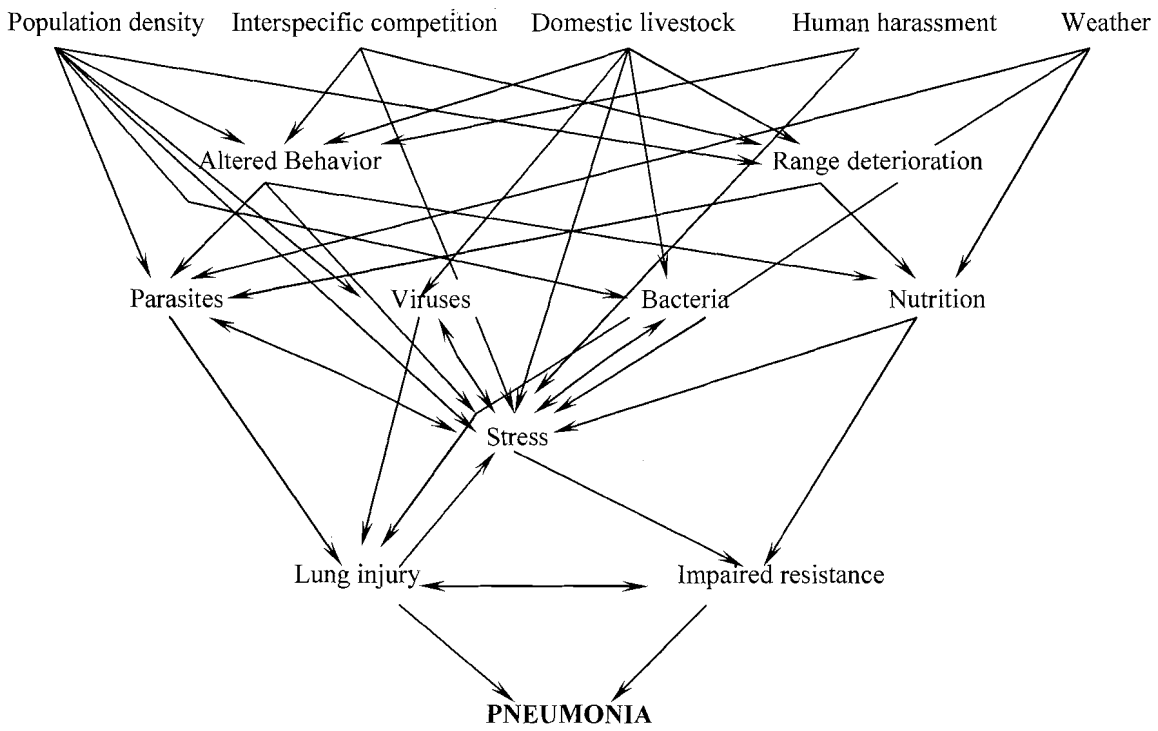
Idaho has experienced similar die-offs in several bighorn sheep populations including the 1988-1990, and 1995-1996 die-off's in central Idaho in the sheep herds of the Salmon River drainage and more recently the Hells Canyon die-off on the Snake River (Dunbar, 1990; Akenson, 1992; Cassirer et al., 1996). Both die-offs had 80-90% mortalities with *P. haemolytica* and *P. multocida* as the bacterial pathogens causing pneumonia. Prior to the most recent outbreaks, the sheep in the Salmon river drainage were said to have had a mass die-off in 1905-1906 which was attributed to the lungworm-pneumonia complex and in the 1950's when a smaller die-off occurred on the East Fork of the Salmon River (Buechner, 1960).

In many of these incidents, domestic sheep were blamed as the primary pathogen carrier. It has been shown that domestic sheep carry strains of *Pasteurella* that kill bighorn sheep while not affecting domestic sheep (Foreyt and Jessup, 1982; Onderka and Wishart, 1988; Foreyt, 1989; Callan et al., 1991). However, there are die-offs that occur without the presence of domestic sheep populations (Buechner, 1960; Wishart et al., 1980; Bailey, 1986; Miller et al., 1991; Aune et al. 1998) and may pose a greater threat to native sheep populations. This is because we have neither easy explanations for their cause nor solutions for their cure.

Factors or stresses contributing to bighorn respiratory disease other than bacteria carried by domestic livestock include weather, interspecific competition, nutrition, viruses, parasites, population density, human harassment and indigenous bacteria (Fig. 1.1) (Wobeser, 1994). These factors can each play an important role alone or in combination in the onset of respiratory disease and will be discussed later. The complex interaction of these factors is what makes management of bighorn sheep populations to

prevent respiratory disease so difficult. Approaches to predicting a die-off begin with identifying the possible stressors associated with each herd and eliminating or managing as many stressors as possible through basic disease management principles. In the simplest form, the primary factors to consider include the host, the agent and the environment. However, there are many approaches within this model that could include management of the causative agent or its vector, manipulation of the host population, treatment and immunization, environmental modification, and influencing human activities (Wobeser, 1994). These options may need to be addressed in order to reduce the chance of a die-off, however, the current condition of disease risk factors and vital statistics of the population must be known in order for them to be addressed efficiently (Downing, 1980).

Fig. 1.1. Schematic diagram to illustrate the interrelatedness of various factors that may be associated with, and form a web of causation for, the "lungworm-pneumonia complex" of wild mountain sheep. Adapted from Wobeser, 1994.



PASTEURELLA

Pasteurella spp. are members of the family Pasteurellaceae and are known to be commensal organisms of ruminants (Magwood et al. 1969; Gilmour et al., 1974; Yates, 1982; Al Sultan and Aitken, 1985; Holt et al., 1994; Queen et al., 1994; Jaworski, 1998). Other members of the Pasteurellaceae family include *Actinobacillus*, *Haemophilus* and *Mannheimia* (Bisgaard, 1995; Angen, et al. 1999). They are non-motile, Gram-negative, facultative and fermentative bacteria that are identified as oxidase positive and produce acid when inoculated into triple-sugar-iron (Becton Dickinson Microbiology Systems) agar slants. The most commonly isolated species of *Pasteurella* from bighorn sheep, as well as other ruminants, are *P. multocida*, *P. haemolytica* and *P. trehalosi* (Ward et al. 1990; Dunbar et al. 1990, Queen et al. 1994; Jaworski et al. 1998; Ward et al. 1997). *Pasteurella haemolytica* that are trehalose negative are currently classified as *Mannheimia haemolytica* (Angen, at el. 1999). However, since the Jaworski et al. (1998) differentiation system is used here to distinguish biovariants, the *Pasteurella* genus will be conserved and *Pasteurella/Mannheimia* (*P./M.*) will depict these organisms.

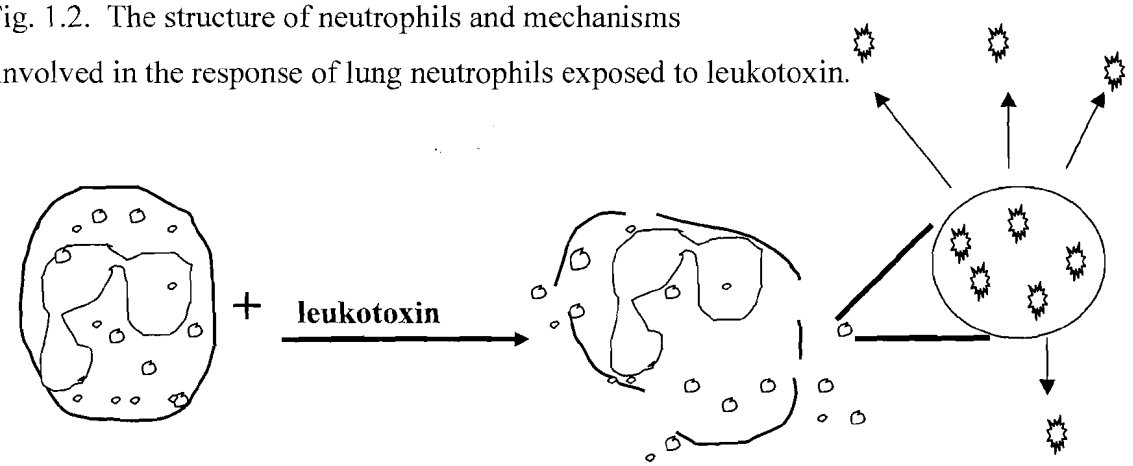
Pasteurella. multocida is an extremely diverse group with multiple strains (Biberstein et al. 1991). It is the primary pathogen involved with fowl cholera, hemorrhagic septicemia in cattle and atrophic rhinitis and pneumonia of pigs (Rimler and Rhoades, 1989; Carter and De Alwis, 1989; Chanter and Rutter, 1989). It is also known to be an opportunistic agent involved with pneumonia of cattle (shipping fever complex) and domestic sheep (Frank, 1989; Gilmour and Gilmour, 1989; Mosier, 1997). The pathogenicity of *P. multocida* depends on the organisms ability to produce lipopolysaccaride (LPS) and protein toxins. Protein toxins are the major factors associates with disease and are produced by some serogroup A and D isolates (Rimler

and Rhoades, 1989). The toxins can produce both hemorrhage and dermonecrosis in a host when the bacteria colonize the critical tissues (Rimler and Rhoades, 1989).

The pneumonia complex of domestic cattle and sheep can be associated with both *P. multocida* and *P. haemolytica* but *P. haemolytica* strains are most often isolated from cases in North America (Collier, 1968; Carter, 1973; Gilmour and Gilmour, 1989). *P. trehalosi*, which shares toxic factors with *P. haemolytica,* has historically been identified as *P. haemolytica* type T (Fraser et al., 1982) until more recent studies have determined it to be a distinct species (Sneath and Stevens, 1990).

Pasteurella haemolytica and *P. trehalosi* have four virulence factors important to the culmination of disease; fimbria, a polysaccharide capsule, endotoxin or lipopolysaccharide (LPS) and leukotoxin (Adlam, 1989). Leukotoxin, a secreted protein with the lytic component having a molecular weight of 105 kDa (Chang, et al., 1987), is the virulence factor thought to be most important in the development of pneumonia in ruminants (Frank, 1989, Gilmour and Gilmour, 1989). Leukotoxin from *P. haemolytica* and *P. trehalosi* has been shown to be specific for bovine and ovine phagocytes (Kaehler et al., 1980; Shewen and Wilkie, 1982; Sutherland et al., 1983). Leukotoxin lyses alveolar macrophages and neutrophils, releasing toxic chemicals designed to kill phagocytized bacteria. Acid hydrolase, muramidase, and myeloperoxidase are all examples of the toxic chemicals, or intracellular enzymes of phagocytes (Green, et al. 1977). As these toxins do not distinguish host cells from bacteria, lytic damage to the host tissue by these released granules contributes to the pathogenesis (Frank, 1989; Green et al., 1977) (Fig 1.2).

Fig. 1.2. The structure of neutrophils and mechanisms involved in the response of lung neutrophils exposed to leukotoxin.



A normal neutrophil with multilobed nucleus and granules containing acid hydrolases, myeloperoxidase, and muramidase. Granule enzymes function to lyse bacterial cells once phagocytized.

After leukotoxin introduction, the cell membrane is lysed, releasing the granules.....

and their contents into the lung where host tissue is exposed to the toxic chemicals.

The *P. haemolytica* leukotoxin protein is controlled by the leukotoxin gene cluster lktCABD, while the active protein is commonly referred to as lktA (Highlander et al., 1990). Expression and regulation of the leukotoxin is not well understood and only partially characterized.

Pasteurella haemolytica and *P. trehalosi* have been characterized as biotypes and biogroups. There are 2 biotypes (A and 3) and 11 biogroups (1, 3, 5, 6, 7, 8, 9, 10, 11, 16, and U) recognized for *P. haemolytica* and 2 biogroups (2, 4) for *P. trehalosi* (Bisgaard and Mutters, 1986). Serotyping was also used to distinguish strains of *P./M. haemolytica* and *P. multocida* isolates (Carter, 1955; Carter, 1956). Seventeen serotypes were eventually identified with types 3, 4, 10, and 15 within biotype T (now known as *P. trehalosi*) and all others representing biotype A and 3 (Frank and Wessman, 1978). Both methods are currently used to distinguish between types of *Pasteurella*.

Further characterization of the Bisgaard and Mutters (1986) biogroups has been established by a set of fermentation patterns for various sugars and enzymes (Fig.1.3). Variations from the 11 strict biogroups are allowed and were characterized by Jaworski et al., (1998) as biovariants (Fig.1.3). Some biovariants have been shown to have host predilection. Jaworski et al. (1998) found that 54% of isolates from wildlife were identified as biogroup 2 biovariants of *P. trehalosi* while only 17% of isolates from domestic animals were *P. trehalosi* variants. In domestic ruminants, 43% of isolates were identified as biogroup 1 biovariants of *P. haemolytica* and only 6% of isolates from wildlife were biogroup 1 biovariants.

Fig.1.3. Criteria for classifying indole negative Pasteurella (*P. haemolytica* and *P. trehalosi*) isolates into biogroups and biogroup variants where brackets indicate the variance in fermentation.
Adapted from Jaworski et al., 1998.

BIOGROUP	TRE	XYL	ARA	SAL	SORB	CELL	MALT	ORN	βGLU	αFUC
16	-	-	-	-	(+)*	-	(+)	-	-	(+)
8	-	+	-	-	-	-	(+)	-	-	-
1	-	+	-	-	((+))	-	(+)	-	-	((+))
3	-	+	-	+	(+)	(+)	(+)	-	(+)	(+)
11	-	+	-	(+)	(+)	(+)	(+)	+	(+)	(+)
7	-	(+)	+	-	-	-	(+)	-	-	-
UT27	-	+	+	-	+	-	(+)	-	(+)	-
10	-	(+)	+	+	(+)	(+)	(+)	-	(+)	(+)
5	-	+	+	((+))	(+)	((+))	(+)	+	(+)	(+)
6	-	(+)	+	-	-	-	(+)	+	-	(+)
9	-	+	+	-	+	-	(+)	+	(+)	(+)
2	+	-	-	(+)	(+)	(+)	(+)	-	+	-
4	+	-	-	(+)	(+)	(+)	(+)	-	-	-

* Parentheses depict specific test results that are allowed to be negative. Double parentheses indicate tests where a negative test, combined with any other negative test, would not be acceptable. Negative tests in one or more of the acceptable locations indicate biovariants.

VIRUSES

Viral infection has been shown to be a predisposing factor in pneumonic pasteurellosis in domestic animals (Carter, 1973; Mosier, 1997). Viruses and evidence of viral infection have been associated with bighorn respiratory disease as well (Parks, et al., 1972; Spraker et al., 1984; Cassirer et al., 1996; Schwantje, 1988). In typical cases of domestic respiratory disease or shipping fever, respiratory viruses and stress from exposure or crowded conditions allows colonization of *Pasteurella* organisms in the lungs and subsequent pneumonia (Carter, 1973; Gates, 1994; Mosier, 1997).

A virus is essentially DNA or RNA within a protein shell (Champoux, 1994). Viruses are intracellular parasites that require the mechanisms of cells in order to reproduce presenting unique problems as it is difficult to target the virus within the host cell when applying therapy (Champoux, 1994). They are also extremely diverse in structure and method of reproduction adding to the complexity of their effects (Champoux, 1994). Many viruses have been implicated in pneumonic pasteurellosis including bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI_3), infectious bovine rhinotracheitis (IBR), bluetongue (BT), adenovirus and bovine viral diarrhea (BVD) (Parks, et al., 1972; Carter, 1973; Spraker, et al., 1985).

Viral infection begins with the adsorption of the virus into a cell (Champoux, 1994). Viral infection is generally limited by the host, where specific viruses can only infect specific hosts (Champoux, 1994). Once inside the cell, the virus uses the cells replication in order to reproduce, in turn killing the cell (Champoux, 1994). All of the various mechanisms by which a virus enters a cell, reproduces and then leaves the cell

contribute to the effects viral infection will have on the host (Champoux, 1994). Viral infections may suppress immune function through multiple mechanisms and in turn predisposes animals to further opportunistic infections (Wainberg and Mills, 1985). Some of those mechanisms include inhibitory factors (prostaglandin) from macrophages, interference of T-cell growth factor synthesis, and activation of suppressor T-cells (Herscowitz, 1985; Wainberg and Mills, 1985). Suppression of pulmonary antibacterial defenses by viral infection includes progression of edema, necrosis and desquamation of epithelium that impairs the mechanical transport of bacteria out of the lung (Green et al., 1977). Direct inhibition of alveolar phagocytes includes suppression of anti-bacterial activity in macrophages that have engulfed bacteria (Green et al., 1977). While many of the mechanisms of viral immune suppression are not well understood, viruses are capable of suppressing the immune system and that suppression may play an integral role in the development of bacterial pneumonia in many species including bighorn sheep.

PARASITES

Parasites, primarily lungworms, have long been implicated in the pneumonia complex. The most common lungworms of bighorn sheep are *Protostrongylus* spp, rather than *Meullerius* spp. or *Dictyocaulus* spp. (Forrester, 1971). The probable life-cycle of the *Protostrongylus* lungworm in bighorn sheep begins with adult nematodes in the lungs, to first stage larvae shed in fecal droppings, land snails as intermediate host, and infective larvae back to bighorn sheep through foraging (Forrester, 1971). Lungworm infection at low or moderate rates likely does not cause any symptoms in normal bighorn sheep and would rarely lead to death (Thorne et al., 1982). Infection of bighorn sheep has been found to fluctuate from year to year and among age classes of sheep (Forrester and Senger, 1964; Arnett et al. 1993). Lungworm infections were found to be higher in lambs than in adults in the same population (Arnett et al., 1993). Reasons for the fluctuations include differences in land snail frequency due to weather conditions or density of bighorn sheep on their ranges, as well as the influence of transplacental infection of lambs (Forreseter and Senger, 1964; Arnett et al., 1993).

One species of lungworm, *Protosrongylus stilesi,* is capable of placental transmission and is important in recruitment of bighorn populations (Thorne et al., 1982). Summer pneumonia is the term applied to lamb pneumonia possibly related to transplacental transmission of lungworms, and may be more common than is currently thought. In Montana, Forrester and Senger (1964) surveyed ten bighorn herds and found *P. stilesi* infection in all ten with *P. rushi* less common (6 of 10). High density *P. stilesi* infections combined with weather, migratory or predator stress and opportunistic bacteria may all contribute to early lamb mortality due to pneumonia (Thorne et al., 1982).

NUTRITION

The study of nutrition of wild animals has been a recent development and most studies are based in food habits (Robbins, 1993). Although the importance of nutrition in prevention of disease has been noted for some time (Chandra and Newberne, 1977), the capability of biologists to prevent an illness in wild animals is minimal when conventional therapy is applied (Cassirer et al., 1996; Foreyt, 1998). Therefore nutritional management as a tool to reduce disease incidence needs to be explored by biologists and researchers.

Nutrition has long been recognized as an important factor of immune function in humans with deficiencies of many single elements capable of causing immunosuppression (Chandra, 1988). Deficiencies of protein, energy, iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), vitamin E, and molybdenum (Mo) have all been associated with poor immune function and increased susceptibility to disease (Chandra, 1988; NRC, 1983). The high occurrence of mineral deficiency associated with immune system depression is easily understood when the distribution of minerals in body enzyme systems is taken into account. There are numerous enzyme-mineral systems within the animal body, many of which are directly or indirectly involved with immune system cells (Chandra, 1988). Nutritional deficiencies in the diet or complicated nutrient interactions of dietary components can effect these systems as well as causing other nutritional complications (Chandra, 1988).

Minimally, all animals, including bighorn sheep, need to take in adequate quantities of feed for protein and energy maintenance to survive. Annual bighorn sheep dietary profiles are similar to those of other wild ungulates as they undergo times of

protein and energy stress in winter and enjoy periods of rapid weight gain and nutritional replenishment in late spring and early summer (Van Soest, 1994; Hebert, 1973). Behavioral adaptations have allowed bighorns to increase their summer nutrient intake even more than expected by normal spring green-up. Hebert, (1973) demonstrated that bighorn sheep, through seasonal altitudinal migration, can increase the amount of digestible nutrients in their diets for extended periods of time. This is through use of alpine community plants that mature later in the year and are therefore in a succulent and usually more digestible growth stage later when compared to plants at lower elevations. Hebert demonstrated that bighorn sheep time their migration so they arrive on summer range just after the plants have begun to green-up which coincides with the reduction of winter range plant quality. This allows them to extend the time they consume high quality forages. By late August, winter range plants in the study had lost much of their nutrient value and had begun to be more fibrous and less digestible. Digestible energy (DE) values of winter range plants ranged from 509.6 kcal/day in June to 410.6 kcal/d by August. However, alpine range plants were still highly nutritious due to the later growing season having DE values of 600.7 kcal/d in August. Feed intake, crude protein (CP) intake, DE intake and average body weights of sheep were all higher for the migratory group of sheep from July through September than for the control group, which stayed on the winter range, during that time. Forages cut from summer and winter ranges at concurrent times were analyzed for protein and energy content. Alpine range plants had consistently higher amounts of CP (15.08 vs 5.97 %) and gross energy (GE) (4.42 vs 4.18 Kcal/g).

Hebert's data provides good evidence that bighorn sheep are capable of taking in adequate nutrients, especially protein and energy, through their migration habits. However, this information does not complete the nutritional profiles of bighorn sheep, nor does it account for variations in different bighorn sheep ranges. While knowledge of protein and energy balances are the first steps in understanding the nutritional state of an animal there are other factors that need to be considered.

First, not all bighorn sheep herds migrate to alpine summer ranges. This may be due to various factors including human blockage of normal migration routes, open ewes with no motivation to move, changes in behavior passed through generations of ewes that do not migrate, etc. (Smith, 1954; Geist, 1971; Spraker et al., 1984). When sheep do not move from their winter ranges, it will be reflected in their total nutritional profile (Hebert, 1973). Lower CP and DE in the diet, decreased intake, and increased fiber are all associated with normal plant succession in herbivore diets (Van Soest, 1994). Bighorn sheep remaining on their winter range will experience gradual decreases in total dietary value as the year progresses. Also, the quantity of forage available in the winter will be reduced by summer utilization. These factors may reduce the overall condition of the sheep making them more susceptible to disease since poor protein nutrition will decrease immune system function (Chandra, 1988).

Second, there has been little analysis of micronutrients including zinc, iron, copper, selenium and others, in bighorn sheep diets. Lack of information on micronutrient content of bighorn sheep diets leaves much room for speculation into the role that micronutrient deficiencies play in the health of bighorn sheep. It has been suggested that nutrition plays a small role in the epidemiology of pneumonic

pasteurellosis due to the rapidness of spread of the disease and multiple cases where the sheep were "healthy", ie. not emaciated (Dunbar, 1992). This cannot be proven however since there is no published report of nutritional analyses of bighorn sheep diets prior to or during pasteurellosis die-offs.

One author, Demarchi, (1968), in addition to protein and energy, reported the fat, fiber, ash, calcium (Ca) and phosphorus (P) for various winter forages utilized by bighorn sheep on one range in British Columbia. This data allows some analysis of the bighorn sheep diet by providing a value for comparison of future dietary analyses. Unfortunately, the lack of information on micronutrients is still limiting to the overall nutritional picture. In addition, the data is really only valid for the geographical location that was analyzed. However, some general statements can be made from this data. For example, Ca and fiber will interact with a variety of essential minerals and the Ca and fiber levels in this diet can be used to determine the amount of these minerals that would be required to meet the animals' needs. Unfortunately, the requirements of these minerals are not known for bighorn sheep so domestic sheep requirements are often substituted as the closest approximation.

Reports on the nutrient content of range forages often utilized by bighorn sheep have been made and can be used to generally assess the forage available to bighorn sheep (Blaisdell et al., 1952; McLean and Tisdale, 1960). However, this data is only an estimate of the nutrients available to bighorn sheep from their diets, particularly because it is necessary to evaluate the diet of each individual herd of bighorns in order to understand the health profile of that herd. A variety of interactions during digestion and

absorption can significantly alter the nutritional value of the animals' diet and each bighorn sheep range is going to vary due to geographic differences.

Some of the more variable and mostly unreported nutrients that are necessary for bighorn sheep immune system health include Fe, Zn, Se, Cu, Mn, and vitamin E (Chandra , 1988; Spears et al., 1991; Stabel et al., 1993; Chew 1994). Iron is a required mineral that is a part of many enzyme systems and is primarily involved with oxygen transport via hemoglobin (Campbell, 1990). Deficiency of Fe has been associated with reduction in the activity of those mettaloenzymes resulting in interference of metabolism in tissues (Chandra, 1988). Iron also plays an important role in the development of young animals. Young born to dams that are deficient in iron have been shown to have liver degeneration and lesions in the spleen and thymus (Chandra, 1988). One of the most important enzymes involved with the immune system is myeloperoxidase. This enzyme facilitates the respiratory burst important for killing of pathogens by phagocytic cells (Ryan, 1994). Reduction in Fe availability caused by a deficiency reduces the ability of both neutrophils and monocytes to kill pathogens through the hydroxyl radical pathway. Increases in disease susceptibility and death have been found in Fe deficient subjects including humans (Chandra, 1994). T cell function has also been found to be depressed by Fe deficiency although the mechanisms are not yet known. Lack of proliferation of T cells is a critical factor in decreased immunity to pathogens as T cells play a central role in antibody production and immune system signaling (Roitt, 1996). Therefore, Fe deficiency may contribute to breakdowns in these two immune system functions during an infection. Since neutrophils and macrophages are the primary cells present in pneumonia lesions, the Fe dietary deficiency may be an underlying cause of

pneumonic pasteurellosis and warrants further attention (Kraabel et al., 1997; Silflow and Foreyt, 1994; Silflow et al., 1993)

Zinc is also part of mettaloenzymes and is required for cellular metabolism and DNA and RNA synthesis (Chandra, 1988; Robbins, 1993). Similar to what is seen with Fe, deficiency of Zn can cause T cell dysfunction and prolonged deficiencies result in thymus reduction. Abnormal immune function, manifested as a decreased cell-mediated response, and increased susceptibility to disease were noted with Zn deficiency and these conditions were found to be reversed with Zn supplementation. Zn deprivation in neonates results in marked reduction in not only body size but in lymphoid organ size as well leading to a lower capacity to respond to immune system stimulation. This low response was associated with the cell-mediated response rather than antibodies or non-specific responses. Low dietary Zn has also been associated with reduced antibody titer response in feedlot steers while Zn supplementation enhanced recovery times in those steers after viral challenge (Chirase et al., 1991; Spears et al., 1991).

Currently, the most widely supported theory about mineral effects on immune system dysfunction involves low dietary Se. Selenium is naturally deficient in many soils around the world and the mountainous northwest is a perfect example (Robbins, 1993). Animals living in this environment, such as bighorn sheep and mountain goats, typically have reduced levels of Se in their blood and tissues as compared to animals in areas where Se is not deficient in the soil (NRC, 1983). Low Se levels are known to be responsible for the white muscle disease pathology, which involves a deficiency of glutathione peroxidase (GSHpx), in both domestic and wild animals (Gates, 1994; Robbins, 1993). The GSHpx enzyme contains Se and is necessary for maintenance of

cell membranes and serves in an antioxidant capacity (NRC, 1994; Robbins, 1993). Capture myopathy in wild animals occurs when stress induced prolonged contraction of the muscles causes the cells to rupture (Spraker, 1982). Cell rupture occurs because GSHpx is not present in sufficient levels to prevent the oxidation of the cell membranes (Robbins, 1993). Capture myopathy due to Se deficiency is a common occurrence among stressed animals in the northwest (Foreyt, 1998).

As with muscles, GSHpx also maintains the cell membrane integrity of phagocytes. After stimulation by an antigen, the cells create reactive oxygen species necessary for pathogen killing (Ryan, 1994). These reactive species may leak out of the vacuoles within the cell and disrupt the phagocytes own membrane (Chandra, 1988). Superoxide dismutase, catalase, and GSHpx are the major systems for controlling this damage (Chandra, 1988). Reduced Se, and therefore GSHpx activity, decreases the ability of neutrophils to handle toxic damage and further reduces the ability of the immune system to respond to immediate infection. Experiments in cattle and rats show reduced non-specific immune response and killing ability in Se deficient animals when compared to Se adequate animals (Chandra, 1988).

Se also works in conjunction with vitamin E to maintain cell membrane integrity. Vitamin E is known to be essential for all cells and is another antioxidant (Robbins, 1993). Vitamin E acts to maintain lipid fluidity of the membrane by protecting it from free radical peroxidation (Chandra, 1988). The importance of the antioxidant capability of vitamin E is mostly noted in the macrophages of the immune system. When vitamin E is low, the cell receptors in the membrane become altered which impairs their ability to recognize foreign antigens or to signal other cells (Chandra, 1988). High vitamin E

levels have also been found to correspond with increased lymphocyte activity in vitro while lipid peroxidation is associated with decreased ability of lymphocytes to undergo mitogenesis (Chandra, 1988). Together, vitamin E and Se are integral for maintaining cell membrane integrity and the function of lymphocytes and phagocytes; in short, the entire immune response.

Interestingly, data is available on the ability of neutrophils from free-ranging bighorn sheep to respond to antigen. Bighorn sheep neutrophils were shown to be more susceptible to killing by *P. haemolytica* leukotoxin than domestic sheep neutrophils and were even more sensitive after being exposed to high cortisol levels, which are present during times of stress (Kraabel et al., 1998). Silflow et al., (1993) also demonstrated the increased susceptibility of bighorn sheep neutrophils to *P. haemolytica* leukotoxin where three to seven times higher killing rates were recorded for bighorn neutrophils over domestic sheep neutrophils subjected to the same leukotoxin. The dietary levels of Se, Zn, Fe, or other important vitamins or minerals were not reported in the animals in these studies. These data along with data on the effects of low Se, Fe, and Zn on the immune system suggest an interaction of poor mineral nutrition in bighorn sheep and subsequent poor immune function.

Only limited data on the circulating blood levels of Se, Zn and Fe can be found. Adequate blood levels for domestic sheep range from 0.12-0.5 *u*g/g. Serum Se values however do not necessarily reflect the amount ingested in the diet and dietary Se values do not correspond to GSHpx activity so conclusions about dietary adequacy can not be made.

Samson et al., (1989) provide the only report relating dietary Se levels to GSHpx activity in bighorn sheep. A significant positive relationship between blood Se values and GSHpx activity was seen when values from different herds were pooled. The hypothesis (Samson et al., 1989) that bighorn sheep were more efficient at converting Se to GSHpx when compared to domestic cattle was not supported by this study. This is meaningful data since the level of GSHpx production and activity may depend on the selenium available in the diet and dietary requirements of bighorn sheep may be more closely related to domestic sheep and cattle requirements than currently hypothesized. This discovery seems likely considering the occurrence of capture myopathy in bighorn sheep and the relationship of low dietary Se to that syndrome. It is ambiguous to agree that low Se contributes to capture myopathy and not also to immune system dysfunction.

Evidence of low mineral status and poor nutrition in bighorn sheep populations and knowledge that nutritional deficiencies cause immune system dysfunction suggests that there may be a link between the incidence of pneumonic pasteurellosis and nutrition in bighorn sheep. However, nutrition is not the only factor involved in any infection and especially not in pasteurellosis. The virulence of the infecting organisms, environmental factors, parasite loads, viral infection as well as other factors can be involved. However, it may be the nutritional state of the animal that allows the disease to progress beyond a mild infection into pneumonia. Much work still needs to be done to understand the importance of nutrition in disease resistance in bighorn sheep.

STRESS

Stress induced immunosuppression is one hypothesis agreed upon by bighorn sheep researchers as a possible cause of pasteurellosis (Thorne, 1982; Spraker, et al., 1984; Bailey, 1986). However, researchers do not agree on the "stress-factor" that is the direct cause. Stress is known to have adverse affects on the immune system and its function in many animals. Stress in animals is said to be abnormal adjustments in physiology or behavior in order to cope with adverse changes and is often measured by increased glucocorticoids (Harlow et al., 1987). Stress can actually cause changes in tissues and organs that can lead to disease pathogenesis and can come from the disease causing agent itself or outside sources (Breazile, 1988). Glucocorticoids also play a role in immune system suppression, limiting inflammatory responses to infection to minimize tissue damage, (Balow et al., 1975; Breazile, 1988). Adrenocorticotrophic hormone (ACTH) is the primary messenger to the adrenal cortex to release glucocorticoids and is stimulated by monocytes, macrophages and activated lymphocytes creating a link between infection and stress (Breazile, 1988).

Normal blood cortisol levels for bighorn sheep have been estimated between 3 and 10 ng/ml (Spraker et al., 1984; Harlow et al., 1987). Harlow also showed increased blood cortisol levels (as much as 30 ng/ml) immediately after exposure to stress (unfamiliar surroundings, handling) that correlated increased cortisol with increased heart rates. Increased cortisol levels (up to 50.6 ng/ml) were also documented in wild sheep during a stress related die-off in Colorado where sheep were constantly exposed to heavy machinery during dam construction within their habitat (Spraker et al., 1984). Stress also increases neutrophil susceptibility to leukotoxin produced by *Pasteurella* spp. (Kraabel,

1997). Since leukotoxin is a very important pathogenic mechanism for *Pasteurella* spp., it seems imperative to understand the relationship between stress and leukotoxin induced neutrophil activity. Neutrophils can cause increased damage to pulmonary tissue through the secretion of free radicals (Breazile, 1988, Roitt et al., 1996). Glutathione peroxidase, requiring Se as a component, is the major defense of cells against neutrophil damage and increased hemorrhage by free radicals (Breazile, 1988; Nockels, 1996).

Factors that cause stress may be difficult to quantify or identify in wild bighorn sheep, however breeding or rut, adverse weather such as prolonged winter storms, poor habitat conditions, and predators are all possibilities. Other stressors have been identified in human activity and vehicular traffic, grazing competition by livestock, high population density, and poor nutrition (Spraker, et al., 1984; Festa-Bianchet, 1988).

It is likely that all of the factors discussed here contribute to stress in some way, making the significance of the stress response extremely difficult to measure. While general stress likely has contributed to bighorn sheep die-offs and pneumonic pasteurellosis, all of the risk factors must be considered when evaluating the cause of these epizootics, and to provide answers for preventing future die-offs.

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Herd Health and Habitat Quality in Relationship to *Pasteurella* spp Induced Pneumonia in Rocky Mountain Bighorn Sheep (*Ovis canadensis canadensis*)

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ABSTRACT

Multiple health-associated factors have been identified as critical elements of the *Pasteurella haemolytica* pneumonia complex in Rocky Mountain bighorn sheep. In order to develop management strategies for bighorn sheep populations, we surveyed two discreet sheep populations in Big Creek, Idaho (BC), and Spanish Peaks, Montana (SP) for bacteria (*Pasteurella* spp), respiratory viruses, lungworm (*Protostongylus* spp), nutritional status and forage nutrient availability. Differences in virulence factors attributed to *Pasteurella* spp as well as differences in predisposing factors were evaluated and compared. Significant differences ($p < 0.01$) in virulence factors for *Pasteurella* spp were seen between the BC and SP populations. In the BC herd, 64% of isolates cultured were β-hemolytic and 38% of those were greater than 50% toxic to BL3 cells. In the SP herd, only 19% of the isolates were β-hemolytic and none of those were greater than 50% toxic to BL3 cells. Serologic evidence did not indicate that either herd had been recently affected by any of the respiratory pathogens, however the BCO herd had a significantly higher titer to BRSV ($p = 0.06$) than the other two herds. Very low lungworm loads (less than 100 LPG) were detected in both herds. Forage nutrient values were similar between the populations as well, however, levels of Se were significantly higher ($p = 0.06$) in the SP population than in BC. In conclusion, it appears that virulence factors of the *P/M. haemolytica* or *P. trehalosi* bacteria may be a better predictor for pasteurellosis, or a *Pasteurella* associated die-off than the presence of these bacteria alone.

Key Words: *Ovis canadensis*, *Pasteurella* sp, pneumonia, ruminant nutrition, respiratory virus

INTRODUCTION

Respiratory disease has been a major mortality factor in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) populations in the northwest and has been implicated as the major cause of sheep declines since the 1800's (Rush, 1927; Buechner, 1960; Post, 1962; Stelfox, 1971; Spraker et al.,1984; Schwantje, 1988). Disease epizootics can be rapid and affect all ages of animals resulting in massive die-offs as well as reducing lamb recruitment for years afterward (Potts, 1937, Marsh, 1938; Post, 1962; Spraker and Hibler, 1982; Thorne et al., 1982).

Pasteurella have been implicated as an important pathogen associated with pneumonia epizootics in bighorn sheep (Potts, 1938; Thorne et al, 1982; Schwantje, 1988; Silflow et al. 1993). A portion of the organisms previously identified in the genus *Pasteurella* have been assigned to the new genus *Mannheimia* using 16S rRNA sequencing (Angen et al., 1999). In addition to *Pasteurella,* other factors are thought to contribute to the development of pneumonia including viral infections, parasite loads, adverse weather conditions, poor nutrition, overt stress and competition from other ungulates (Buechner, 1960; Parks, et al., 1972; McCoullough et al., 1980; Thorne, et al., 1982; Spraker, et al., 1984; Spraker and Collins, 1986; Dunbar, 1992; Stabel, et al., 1993).

In most cases, investigation into herd health takes place after a die-off or decline has occurred as a means to diagnose and chronicle the course of the disease. However, there is no treatment for pneumonic pasteurellosis in free-ranging bighorn sheep, and a better way to mediate its effects may be through proactive management. This might include periodic monitoring of herds for not only population management factors but also for risk factors associated with pasteurellosis, as well as creating trigger values for mitigation events.

The objectives of this study included:

1. Evaluation of pharyngeal swabs collected from bighorn sheep for *Pasteurella* spp and viruses.
2. Evaluation of feces for intestinal parasites.
3. Collection of blood samples for chemistry profiles and serum antibody quantitation.
4. Evaluation of forage available for and utilized by bighorn sheep in test populations.
5. Compare results of evaluations for the Spanish Peaks population with those from bighorn sheep in the Big Creek herd of Central Idaho which has a history of respiratory disease.

This study evaluated samples from two bighorn populations collected between September 1997 and May 2000. One population (BC) was recovering from a major pneumonia die-off that occurred from 1988-1990, and another (SP) had no history of pneumonic pasteurellosis. Data collected during the study was compared to data collected from bighorn sheep during the die-off of 1988-1990 (BCO). This information was obtained from the Idaho Department of Fish & Game Wildlife Laboratory records. From 1997 to 2000, bacteriology, virology, serology, blood chemistries, parasitology and diet nutrient analysis, were performed concurrently in both populations to determine the health status of each population and look for differences that might alert game managers of susceptibility to pneumonia. Evaluation of the results and comparison of health profiles may help identify risk factors associated with pneumonic pasteurellosis in bighorn sheep and provide managers insight for proactive health management.

MATERIALS AND METHODS

Sheep:

Free-ranging Rocky Mountain bighorn sheep were sampled from two discreet populations: 14 (2 rams, 7 ewes, 5 lambs) from the Big Creek drainage population of central ID, UTM 5000.0 N x 659.0 E x 4994.0 N x 677.0 E and 15 rams from the Spanish Peaks, MT population, UTM 5033.0 N x 454.0 E x 5012.0 N x 486.0 E. Big Creek sheep were captured by either immobilization (n=5) with a combination of Xylazine/Ketamine (dosage: 1.5 mg/kg zylazine, 100mg/ml and 7.5mg/kg ketamine, 200 mg/ml) or Telazol/Zylazine (rehydrated Telazol with 100mg/ml Xylazine to 120 mg/ml; dose: 1ml/75 lbs), or by helicopter drive netting (n=9) from December 1998-April 2000. Chemical immobilization of animals was reversed with Tolazaline at 1.5 ml/75 lbs at least 60 minutes post immobilization. Samples collected from the Spanish Peaks population were from hunter-harvested rams taken during the 1997, 1998 and 1999 hunting seasons and one ram captured by clover trap in February 1999. The animals were examined for condition, external parasites, and injuries. Nasal (NA), oropharyngeal (OP), blood or vitreous humor, and fecal samples were collected from each of the animals.

Bacteriology:

Oropharyngeal samples were obtained using Accu-Culshure (Accu-Med Corporation, Pleasantville, New York, USA) collection systems from live animals (BC), and Precision Culture Collection and Transport System with charcoal Amies modified medium (Precision Dynamics Corporation, San Fernando, California) from hunter-harvested animals (SP). Samples were sent to Caine Veterinary Teaching Center (CVTC) where they were inoculated onto Columbian blood agar with 5% ovine blood (CBA) and a CBA plus antibiotics (CBAA) selective for Pasteurellaceae (Jaworski et al., 1993). Inoculated media were placed in a 37°C

incubator with 10% CO_2 for 24 hours. Cultures were examined for typical Pasteurellaceae colonies and a representative of each such colony was selected for further characterization. Color, size, texture and hemolytic activity were evaluated for each selected colony. Isolates with phenotypic characteristics listed by the Center for Disease Control and Prevention for *P. haemolytica* (Weyant, et al. 1995), a portion of which now identified in the genus *Mannheimia* (Angen et al., 1999), were tested further for identification of biovariants by the procedures of Jaworski, et al., (1998). Isolates previously identified as *P. haemolytica* were identified as *P./M. haemolytica* followed by biovariant notation.

All *P./M. haemlotyica and P. trehalosi* species were biogrouped and the presence of the leukotoxin gene (*lkt*A) was recorded as *lkt*A is the structured gene for a major virulence factor (leukotoxin). The cytotoxicity of isolates with the *lktA* gene was also quantified to estimate the potential virulence of each isolate. Additional substrates were used to test indole-positive isolates (Biberstein et al., 1991). Isolates of *P./M. haemolytica* from the BCO period were archived at CVTC, and were compared to the current isolates.

Restriction Enzyme Analysis (REA) and DNA Isolation:

Pasteurella/Mannheimia isolates from both bighorn sheep populations that were determined to be biochemically identical were subjected to REA to determine relatedness. Isolation of DNA for REA analysis was conducted using the Gentra Puregene DNA isolation kit (Gentra Systems Inc., Minneapolis, MN). DNA was quantified by flourometry and a standard of 6.0 ug per reaction were cut with *Hae*III restriction endonuclease (10 U) in a 60ul reaction for 2.5 hrs at 37°C. Restriction digests (20ul of reaction volume) for each isolate were loaded into wells of a 1% agarose gel and run for 16 hours at 30 volts. Standard 1 kilobase (kb) ladders were used as base pair markers. The gels were stained for 1 hour in ethidium bromide and viewed with a Bio-Rad Gel Doc 2000 gel imager (Bio-Rad

Laboratories, Hercules, California). Restriction fragment banding patterns were used to determine relationship indices between isolates from each herd by biotype (Nei and Li, 1979). Dendograms were created with the unweighed pair-group method using arithmetic averages (UPGAMA) cluster analysis (Sneath and Sokal, 1973).

Polymerase Chain reaction (PCR):

PCR was used on all isolates to detect the presence of the *lkt*A gene. Amplification of the *lkt*A gene was done following the methods of Fisher, et al., (1999). Briefly, to a 50 ul reaction volume of 21.5 ul water, 25 ul 2X reaction buffer, 1ul of each primer and 1.5 ul Red Taq (Sigma Corporation), 1.0 ul of purified DNA was added for each sample and the reaction mixture was placed in a thermocylcer for 30 cycles. The final product (12 ul) was loaded on a 1.1% agarose gel with Sigma PCR marker (Sigma Corporation) and run at 90 V for 45 minutes. Gels were stained one hour in ethidium bromide and viewed with the Bio-Rad Gel Doc 2000 for demonstration of the the *lkt*A gene.

Leukotoxin Analysis:

Hemolytic, *lkt*A positive isolates were cultured and leukotoxin was harvested by the methods of Shewen and Wilkie, 1982. Briefly, isolates were incubated in brain heart infusion (BHI) broth for 16 hours, centrifuged, then resuspended in RPMI 1640 with 7% fetal bovine serum and incubated for one hour for toxin production. Isolates were then centrifuged again at 4°C to stop growth phase of organisms. Three 1ml aliquots of supernatant were filtered through 0.45um syringe filter tips and frozen at 0°C. Harvested supernatants were then used in a colorimetric cytotoxicity assay as described by Greer and Shewen (1986), to determine toxicity. The procedure utilized bovine leukocyte (BL3) cells, which were cultured in RPMI, and suspensions of approximately 1.2 x 10^6 viable cells per ml were prepared. Suspensions (200 ul/well) were dispensed in 96 well tissue culture plates.

The plates were centrifuged (500 x g, 10 min) and 200 ul of each test leukotoxin preparation was distributed in each of 8 replica wells. Plates were incubated one hour at 37°C in CO2. Wells were again centrifuged (500 x g, 10 min) and washed in 200 ul/well saline. Then, 200 ul/well of neutral red working solution was dispensed into wells and plates were again incubated for one hour at 37°C in CO2. After a final centrifuging and two saline washes, cells were lysed with 200 ul lysis solution (50 mM HoAc, 0.5% SDS) and read in an EIA microplate reader at optical density, (OD_{540}). Cytotoxicity was measured as a percent of cells killed by the following calculation.

$$\% \text{ Toxicity} = \frac{A-B \times 100}{A}$$

Where A= mean OD of control wells; B= mean OD of test wells

The methods of Greer and Shewen, (1986) were used to determine virulence where toxicity over 50% is considered virulent and less than 50% avirulent. A chi square test was used to test for differences in toxicity of biovariants between the herds (Moore and McCabe, 1993).

Serology and Blood Chemistry:

Blood was collected (BC, N=14; SP, N=5) using the Vacutainer system (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) into two 8.5 ml SST gel and clot activator tubes and two sodium heparin tubes per animal. Clotted blood was centrifuged within 24 hours at 2500 rpms for 10 minutes to separate serum. Serum was sent to the Bureau of Animal Health Laboratory, Boise, ID for serologic testing including anaplasmosis, bluetongue (BT), RSV, IBR, BVD, PI_3 epizootic hemorrhagic disease (EHD), *Brucella ovis*, leptospira, and ovine progressive pneumonia (OPP). Heparinized blood was sent to the

University of Idaho Holm Research Center, Moscow, ID for quantification of selenium (Se) levels. Differences in means between the populations were tested with analysis of variance. Titer data was transformed to $\log_2$ before analysis (Snedecor, 1946).

Vitreous humor, collected by aspiration from the eye of seven hunter-harvested bighorns in MT, and blood from all other animals were sent to the Treasure Valley Laboratory, Boise, ID for chemistry analyses. These included tests to quantitate values for glucose, blood urea nitrogen (BUN), creatinine, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorus (P), iron (Fe), total protein, albumin, globulin, alkaline phosphatase, SGOT, GGTP, total bilirubin, triglycerides, cholesterol, and selenium (Se). Blood chemistries from each herd were pooled for each test and evaluated on a herd level basis.

Virology:

Nasal samples were collected using Viral Culturette swab systems which contained 0.5 ml modified Hank's balanced salt solution (HBSS) (Becton Dickinson & Co., Cockeysville, Maryland) and were sent to CVTC where they were inoculated into appropriate cells for viral culture. Briefly, swabs were washed in 2mls HBSS. Inoculum (200 ul/well) was pipetted onto 24 well tissue culture plates containing 24 hour monolayers of bovine turbinate (BT) cells, bighorn sheep turbinate (BHST) cells, and bighorn sheep kidney (BHSK) cells (DeLong et al. 2000). Cell cultures were incubated for 10 days and observed for cytopathology. Cultures were passed two additional times and stained with fluorescent labeled antisera to bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI_3), bovine respiratory syncytial virus (BRSV) and adenoviruses.

Parasitology:

Fecal samples were extracted from the rectum of captured and killed animals (BC, n=14; SP, n=5) and placed in a Whirl-pak (Nasco Corporation) bag for parasitology. The primary

parasite of interest was *Protostrongylus* spp., however other species included nemotodirus, coccidia and trichuris. Random fecal samples were also obtained after defecation during periods of bighorn observation (BC, N=14; SP, N=13). The Baermann (1917) procedure for *Protostongylus* larvae was performed at CVTC and Washington State University. Baermann results from all animals were combined for each population and were evaluated against the Thorne et al. 1982 method for lungworm load analysis where infections of fewer than 500 LPG (larvae per gram) are considered low infections.

Forage and Habitat Evaluation:

To determine the nutritional quality of the diets for bighorn sheep on their respective ranges as a measure of nutritional health, samples of both forage available and forage utilized by bighorn sheep were collected. Samples were taken at four intervals representing spring/ lambing (S) range, summer (SU) range, early winter/ breeding (W) range and late winter (W2) range for the Spanish Peaks and Big Creek herds. Available forage was determined by discovery of ranges being used by the sheep at the specified times. Representative areas within the same habitat type predominantly used by the herds were used to gather available forage. Samples were collected using the Daubenmire method (BLM-NARSC, 1996). A total of 12 two ft^2 randomly selected plots were read to identify species and percentage of cover while five (also randomly selected) of those were collected for forage analysis. The same five plots were collected for each interval and for each herd.

Diets were determined through binocular (10 x 25) or scopic (10 x 40) observation of sheep for periods of at least 10 minutes per animal and for one hour per group of sheep. Bites of individual species were recorded and confirmed through close observation of grazing area immediately following a grazing bout to identify freshly bitten plants. Representative samples of the eaten plants were collected and weighed immediately to determine fresh

weights of the forage. Composition of diets was determined by roughly estimating bites per species and obtaining percentages of total bites. Once collected and weighed, all samples were dried at 55°C, reweighed, and ground to 4mm. All samples were then sent to Iowa Testing Laboratories, Inc., Ames, Iowa, USA for total nutrient analysis and selenium testing.

Values were obtained from each bighorn herd for the following parameters: acid digestible fiber (ADF); Calcium (Ca); Copper (Cu); digestible protein; Iron (Fe); metabolizable energy (ME); phosphorous (P); total protein; selenium (Se); total digestible nutrients (TDN); and Zinc (Zn). Differences in means between the two populations were tested with analysis of variance. Percentage data was transformed to arc sine square root before analysis (Snedecor, 1946).

RESULTS

Bacteriology:

Pasteurella/Mannheimia spp were cultured from 12 of 14 (86%) BC bighorn sheep and 11 of 15 (73%) SP bighorn sheep (Table 2.1). Thirteen biovariants of *P./M. haemolytica* and three biovariants of *P. trehalosi* were isolated from BC samples. Of those 16 biovariants, nine or 64% (seven *P./M. haemolytica* and two *P. trehalosi*) were β-hemolytic on CBA. Six biovariants of *P./M. haemolytica*, eight biovariants of *P. trehalosi*, and one isolate of *P. multocida* were isolated from the SP herd (Table 2.1). Only three (19%) of the isolates (one *P./M. haemolytica* and two *P. trehalosi*) were β-hemolytic on CBA. Two *P./M. haemolytica* and two *P. trehalosi* variants were identified during the die-off (BCO) and three (75%) (two *P./M. haemolytica*, one *P. trehalosi*) were β-hemolytic (Table 2.1).

REA and DNA Isolation:

Biochemically similar isolates of each species cultured from the two populations were evaluated by REA. Restricted fragments were similar for biotype and herd as shown in Fig. 2.1. The isolates from the two populations had differing REA fragment patterns but those of the isolates from each herd were similar (Figure 2.1). However, β-hemolytic *P. trehalosi* and non-hemolytic *P. trehalosi* isolates from the BC herd had markedly differing REA fragment patterns. The SP *P./M. haemolytica* isolates had more variable REA fragment patterns than the *P. trehalosi* isolates and had virtually no identical REA fragment patterns (Figure 2.2).

Thirty-two isolates identified from both herds (including biochemically identical isolates from multiple sheep) presented 25 unique REA fragment patterns (Figure 2.3). One isolate (00-608-22), a non-hemolytic *P./M. haemolytica* cultured from a 2.5 year old BC ewe, was distinctly different from all other isolates with a similarity coefficient (SC) of 0.16. This

isolate was the only one of this type cultured from the BC herd and was a biovariant $1^{\alpha B}$. Sixteen isolates were grouped in a second cluster, SC of 0.44, with all of the *Pasteurella* isolates from SP, four *P./M. haemolytica* isolates from BC and two *P. trehalosi* isolates from BCO. A third cluster with SC of 0.40 was comprised entirely of *P. trehalosi* isolates from BC and BCO, with one subcluster (n=3) having an SC of 1.0, and one larger subcluster (n=5) with SC of 0.95. The fourth cluster (SC=.55) is comprised of both *P./M. haemolytica* and *P. trehalosi* from BC and BCO, with the *P./M. haemolytica* isolates all cultured from BC and the *P. trehalosi* isolates all cultured from BCO.

PCR:

Fifteen SP, twelve BCO, and 26 BC isolates, including identical variants from different individual bighorn sheep, were tested by PCR for the *lkt*A gene (Table 2.1). Nineteen (73%) BC isolates were positive, four (15%) weakly positive and three (12%) were negative. Weakly positive reactions had a visible band at 1145bp but were not as distinct as those of positive reactions as demonstrated in Figure 2.4. Of the twelve BCO isolates, 11 (92%) were positive and 1 was negative for the *lkt*A gene. By contrast, of the 15 SP isolates, two (13%) were positive, six (40%) were weakly positive, and seven (47%) were negative for the *lkt*A gene.

Leukotoxin Analysis:

Isolates that were either positive or weak positive for the *lkt*A gene were tested by BL-3 assay for toxin production. Isolates from BC and BCO were significantly more toxic than those from the SP herd ($p < 0.01$). The BC and BCO herd isolates were not different ($p > 0.25$). In the BC herd eight of 21 (38%) *lkt*A positive isolates tested scored above 50% toxicity with a range of 56- 96% toxicity. A *P. trehalosi* isolate scored the highest toxicity of 96%. The mean toxicity for all eight isolates was 74%. In the BCO isolates, four of eight

*lkt*A positive isolates were above 50% toxicity (76-83%). Of these four, the highest toxicity was again a *P. trehalosi* with a mean of 80% toxicity. In the SP herd, none of the isolates tested above 50% toxicity (0-27%) and the mean of all isolates (n = 8) was 7%. The highest toxicity level recorded for the SP herd was from a *P./M. haemolytica* variant.

Serology and Blood Chemistry:

Serologic evidence of exposure to respiratory pathogens indicates a low exposure rate for the BC herd and virtually no exposure in the SP herd (Figure 2.5). One BC 1.5 year old male had a moderate PI_3 titer (1:32) while one 5.5 year old ewe had low titers (1:8) for BVD, BRSV, IBR and PI_3. Also, low titers to anaplasma (= or < 1:5) and leptospira (= < 1:50) were present in three and four animals respectively. No titers to EHD, BT, OPP or *B. ovis* were found in any population. In the SP herd, low titers for PI_3 (1:8); anaplasma (1:5), and leptospira (1:50) were found in one animal for each test. None of the other SP samples tested positive for antibodies against any of the other targeted respiratory pathogens (Figure 2.5). The BCO herd however, had multiple sheep with titers to respiratory viruses including BVD, PI_3, IBR and significant titers to BRSV (P = 0.06) (Table 2.2).

Blood chemistry values for both herds are reported in Table 2.3. Whole blood selenium (Se) levels for the BC and BCO populations ranged from 0.011-0.15 ppm with a mean of 0.045 ppm (n=14) and < 0.01-0.05 with a mean of 0.036 ppm respectively. No blood selenium data was available from the SP herd. Other values were normal for all herds except for Cl, high at 132 meq/l and Ca, low at 7.7 mg/dl in the SP sheep which may be due to vitreous humor testing rather than whole blood.

Virology:

All bighorn sheep samples (BC = 14, BCO = 5, SP = 15) were culture negative for all viruses.

Parasitology:

Baermann test results for *Protostongylus* spp. gave a mean LPG of 23 from BC, with the highest individual shedding 168 LPG, and a mean of 57 LPG from SP, with the highest individual shedding 512 LPG. No individuals had high lungworm loads, and neither herd showed a significant lungworm infection on a pooled basis. No parasitology results were available from individual sheep from BCO. Differential shedding was apparent in the samples for both herds based on time of collection, where those from the summer range (BC = 0.33 LPG; SP = 0.33 LPG) were lower than those from the winter range (BC = 56 LPG; SP = 101 LPG). Lungworm loads were also noticeably higher during late winter periods than early winter (BC = 84 and 29 LPG; SP = 118 and 84 LPG).

Forage and Habitat Evaluation:

Results of forage analyses are presented in Table 2.4. Domestic sheep requirements were used as a model for bighorn sheep as no data on the forage nutrient requirements of bighorns is available. Zinc in ppm ranges from 23 to 41 in BC and from 22 to 33 in SP diets. For the bighorn diets, Ca levels were between 0.49% and 1.3 % for BC and 0.25% and 2.6% in SP at various times of the year. Iron levels ranged from 78 to 1229 ppm in BC and from 106 to 1257 ppm in SP. Copper (Cu) levels for the diets of these two populations average 7.75 ppm in BC and 8.5 ppm in SP. Phosphorous (P) levels were significantly different, ($p = 0.08$), where the BC herd was 0.18 ppm and the SP herd was 0.25 ppm. Levels of Se in the BC diets ranged from 0.012 to 0.05 ppm with a yearly average of 0.027 ppm. SP diets had Se values from 0.03-0.052 ppm and averaged 0.035 ppm. Significant differences were detected in Se between the two populations ($p = 0.06$).

DISCUSSION

Biovariants of *Pasteurella* sp. identified were different between herds and sample collection times. This would be expected due to the variances in exposure, to other wildlife species as well as other bighorn sheep, and mutations over time. Each herd may be considered to have a bacterial flora fingerprint. *Pasteurella/Mannheimia* isolates were not cultured from all animals sampled in either the BC or the SP herd. Although *Pasteurella/Mannheimia* are considered a normal commensal organism of bighorns, (Queen et al. 1989) there are several reasons for the lack of culture from some samples including time of sampling after death, swabbing technique of the tonsillar crypts, and duration of time between sample collection and actual culture due to remote locations. In the BCO herd, even fewer organisms were isolated most likely due to time between death and culture as most samples were opportunistic. In addition to differences between the biovariants of *Pasteurella* sp. found in each herd, the isolates also differed with regard to virulence of those biovariants. This may help to explain the variance in pasteurellosis die-offs between the two herds. The BC herd still carried a highly toxic biovariant of *P. trehalosi* that was initially found during the die-off in 1989 (Jaworski et al., 1993). No highly toxic biovariant was cultured from the SP herd which has never had a pasteurellosis die-off.

The BC herd also carries the *P./M. haemolytica* $1^{\alpha B}$ biovariant. Biovariants of biogroup 1, or the A biotype, are common in domestic sheep (Ward et al., 1990). Biogroup 1 organisms were not cultured from the SP herd. Although the SP bighorn sheep may have had opportunity to commingle with domestic sheep, no specific instance has been reported and biovariants commonly associated with domestic sheep were not isolated from any of the SP bighorn sheep samples.

Pasteurella trehalosi strains are most commonly isolated from wild ruminants. A majority of *P. trehalosi* biovariant 2 isolates were cultured from BC, and these isolates had the highest leukotoxicity (96%). A similar isolate was first identified by Jaworski et al., 1993, from a bighorn ewe captured in the central ID herd. This isolate was thought to be persistent in the herd due to repeated isolation from the herd over a two year period during the die-off. Although many of the SP isolates were weakly positive for the *lkt*A gene by PCR they showed zero or very low toxicity of less than 10% in the BL3 assay. If leukotoxin is a primary factor involved in the development of pneumonia, the difference in toxicity in *Pasteurella* sp. carried by any herd will likely impact the severity of the disease.

. The LKT operon is comprised of 4 genes (*lkt*CABD), with the active protein being referred to as *lkt*A. Fisher showed approximately 95% correlation between *lkt*A and hemolysis. The SP isolates may be weak positive due to less total DNA in the reaction volume or primers not matching 100% due to point mutations in the code (Fisher, 1999). These bands may also represent non-specific binding, which is indicated by the lack of toxicity in these isolates (Erlich, 1992).

Although no viruses were cultured from either herd, and serology indicated low level exposure or infections, the role of viruses in a die-off should still be evaluated. During the die-off in BC, PI_3 and BRSV were both indicated through serology, seven and five of 12 respectively, though no viral cultures were made. Low titers to BVD, IBR, anaplasma, *Leptospira* and *Haemophilus* sp. were also found in a few bighorn sheep. Since no repeated titers were available from any of the bighorn sheep, it was not possible to determine if any of the titers represented new infections or residual antibody from previous exposure. It seems likely with titers to BVD, BRSV, IBR and PI_3 present in the BCO herd, that the older BC ewe was exposed previously and her titers were residual. The 1:32 titer to PI_3 virus detected

in the serum from the 1.5yo male may have resulted from exposure while commingling with other males from other herds, common behavior for bighorn rams in a meta-population (Geist, 1971). Respiratory viruses may play a substantial role in a pneumonia die-off by causing damage to the lung, allowing opportunistic pathogens such as *Pasteurella* sp. to flourish. It is possible that during the 1988-1990 die-off in Big Creek that viral infections were affecting the bighorn population resulting in the elevation in titers detected in the BC serum samples. Combined with a virulent type of *Pasteurella* sp., the immune system was overwhelmed which led to pneumonia. It seems likely that a viral component was a strong factor in the BCO die-off since the BC herd still carries the virulent *Pasteurella trehalosi*, detected during the die-off (Jaworski, 1993), has no evidence of current respiratory viral infection, and has not been affected by a die-off recently.

Further, as the SP bighorn sheep have no serologic evidence of exposure to respiratory pathogens, and have not had a pneumonia epizootic, respiratory pathogens must be considered critically during a die-off. Especially when combined with virulent strains of bacteria. However, other factors may be influencing susceptibility such as selenium in the diet.

Blood Se values for the BC herd were abnormally low. A normal whole blood range for selenium in domestic sheep is 0.05-0.12 ppm (NRC, 1983). Normal blood selenium levels for Rocky Mountain bighorn sheep have not been determined, but have been reported from 0.0005-1.60 ppm (Samson, et al., 1989; Kock et al., 1987). However, low blood SE was expected, as the habitat of Rocky Mountain bighorn sheep is known to be deficient in this mineral. The Rocky Mountains of the northwest U. S. are generally low in Se due to the type of rock formations present (Robbins, 1993). Also, Se is leeched from high mountains to low

valleys and prairies through normal runoff, which contributes to low Se availability in high elevations (Robbins, 1993).

For the BC herd, average BUN was higher than normal (26mg/dl) and the P level was also high (6.2mg/dl) which may have been the contribution of nine individuals that were caught by helicopter drive netting in one day. These nine individuals averaged a BUN of 31 mg/dl, and P of 7.5 mg/dl, which are abnormally high values and probably reflect overall hydration of the herd at the time of capture, or dehydration of individuals during drive-netting operations. With these nine eliminated, the BUN and P for the BC herd are 17.2 and 3.82 respectively, which are normal values. However, there is nothing outstanding in the blood chemistry values to suggest abnormal nutrition of the BC bighorn sheep except low Se, which may play a role in immune system health.

In the SP herd, the Cl value of 132 was abnormally high and may reflect poor Na:Cl balance in these sheep at high elevations. Ca was also low, but may be due to sampling of the vitreous humor rather than the blood. Vitreous humor was readily accessible in most dead bighorns submitted; however, the time before collection after death may contribute to differences in the Ca levels reported (Zaugg and Kinsel; 1997).

Parasitology results indicate no significant *Protostongylus* infection in either herd of bighorn sheep at the present time. While lungworm shedding fluctuated over the year for both herds, neither herd shed more than 500 LPG, and both may be considered to have low infection rates. Fecal rates of 100-500 LPG are considered relatively low (Thorne et al., 1982). Many factors may have contributed to the low counts in each herd including low land snail numbers or low sheep density. These factors may also account for the seasonal variation in lungworm loads where summer range, when sheep are at lower density and higher elevations, have lower counts than winter range. In the SP herd, treatment through

supplemental feeding of fenbendazole pellets was done annually on a small portion of the herd from approximately 1988-1997 (Aune; 1997). This may have had a substantial effect on the lungworm infection in this herd. The BC herd was not supplemented with any kind of de-worming medication at any time. However, the BC herd was also at a low population density compared to numbers prior to the die-off (Akenson and Akenson, 1992).

Forage selenium (Se) requirements of 0.10 to 0.12 ppm are recommended for domestic sheep (NRC, 1985). Whole blood values of 0.2 ppm are recommended for adequate Se transfer to the fetus, a marker for adequate Se levels (Kincaid, 1998). Studies have shown for whole blood Se levels to reach at least 0.2 ppm, dietary Se levels must be between 0.25 and 0.30 ppm (Kincaid, 1998). The whole blood Se values from the BCO (n=5) and BC (n=14) sheep averaged 0.04 and 0.045 ppm respectively. There were no blood Se values available for the SP sheep. The forage selenium levels in diets of both sheep herds are on average 8-10 times lower than recommended (SP=0.035ppm; BC=0.027ppm) and could contribute to the poor immune function of bighorn sheep. Speculation about the requirements of this mineral in bighorn sheep has noted that perhaps animals adapted to the alpine environment do not require as much Se as domestic animals (Samson et al. 1989). This may be true to some extent, however, the levels that are reported here are much lower than one would expect. Bighorn sheep are shown to be more similar to domestic animals than mountain goats in their Se utilization (Koller et al., 1984; Samson et al., 1989), indicating that they most likely require much higher levels of Se in their diet than were found for proper immune function.

Micronutrient interaction in the diets of ruminants can be very complex and nutritional deficiencies can be difficult to diagnose. Ranges of dietary zinc (Zn) deemed adequate for domestic sheep by the NRC, 1985 range from 20 to 30 ppm with a limit of 750 ppm before

there is concern of causing copper deficiency. The Zn levels appear to be adequate, however diets high in Ca can induce Zn deficiency. It is recommended not to exceed 1.2% Ca in the diet as levels above this begin to cause Zn deficiency (NRC, 1985). The SP population may experience a period of Zn deficiency during the summer and early fall because the Zn levels are not high enough to overcome the high Ca in the diet.

Dietary iron (Fe) values for both herds are more than adequate and the high values may reflect aberrant soil that contaminated the samples, but which also gets into actual sheep diets as well. Fe toxicity may occur at a level of 500 ppm, and is recommended not to be exceeded (NRC, 1985). From this data, Fe does not appear to be a limiting factor on the immune system of these two populations of bighorn sheep.

Since the difference in leukotoxicity of *Pasteurella/Mannheimia* between the two herds is significant, one could conclude that virulence of specific commensal organisms contributes highly to the probability that a herd will experience pneumonia epizootics with or without other stress. With the significant differences in selenium also a factor, it is suggestive that micronutrients may have a substantial role in the immune system health of bighorn sheep.

Further comparison of specific risk factors and virulence of *Pasteurella/Mannheimia* biovariants found during die-offs as well as in normal healthy populations is needed to fully understand the dynamics of a *Pasteurella* epizootic in Rocky Mountain bighorn sheep. Better baseline testing of sheep herds at each opportunity is needed to detect changes in specific risk factors within the herds. Also, setting of trigger values that will elicit a response by the managers before a major problem occurs should be considered. Since bighorn sheep herds will vary in their exposures, commensal flora, natural stressors, diet etc., the same trigger values may not be needed for each herd. Testing for lungworm loads, respiratory

viral exposure, *Pasteurella* typing and culture of other respiratory bacteria at a minimum should be considered.

Table 2.1 *Pasteurella* cultured from bighorn sheep from Big Creek, Idaho 1998-2000 (BC), 1988-1990 (BCO) and Spanish Peaks, MT 1997-2000 (SP).

Animal ID	Date collected mo/yr	Sheep Age Sex	*Pasteurella* isolated	Biovariant	Hemolysis	*lktA* (PCR)[1]	%Toxicity	Isolate #
BC1	12/98	Adult[2] ewe	*P. haemolytica*	$9^{\alpha\beta R}$	(β)[3]	+	16	98-1621-10
BC1	12/98		*P. trehalosi*	2	(nh)[4]	+	5	98-1621-3
BC2	3/99	Lamb ram	*P. trehalosi*	2	(β)	+	96	99-302-1
BC3	3/99	Adult ewe	*P. haemolytica*	$1^{\alpha B}$	(β)	+	19	99-319-5
BC3	3/99		*P. trehalosi*	2	(β)	+	56	99-319-1
BC4	4/00	Adult ewe	*P. haemolytica*	3^{BCX}	(β)	-	NT[5]	00-608-9
BC4	4/00		*P. haemolytica*	$9^{\alpha\beta RX}$	(β)	+	43	00-608-92
BC4	4/00		*P. haemolytica*	$1^{\alpha B}$	(β)	NT	NT	00-608-93
BC4	4/00		*P. trehalosi*	4	(β)	+	66	00-608-2
BC4	4/00		*P. trehalosi*	2	(β)	+	72	00-608-94
BC5	4/00	Lamb ram	*P. haemolytica*	8	(β)	+	71	00-608-101
BC6	4/00	2.5 ewe	*P. haemolytica*	$1^{\alpha B}$	(nh)	+	25	00-608-22
BC6	4/00		*P. haemolytica*	$U^{\beta BX}$	(nh)	-	NT	00-608-111
BC6	4/00		*P. trehalosi*	2	(β)	+	56	00-608-113
BC6	4/00		*P. haemolytica*	$1^{\alpha B}$	(β)	+	29	00-608-112
BC7	4/00	1.5 ewe	*P. haemolytica*	3^{BX}	(β)	+	4	00-608-32
BC7	4/00		*P. haemolytica*	$U^{\beta BX}$	(nh)	NT	NT	00-608-121
BC7	4/00		*P. trehalosi*	2	(β)	wk+	48	00-608-31
BC8	4/00	Lamb ewe	*P. haemolytica*	$1^{\alpha B}$	(β)	NT	NT	00-608-42
BC8	4/00		*P. trehalosi*	2	(β)	+	83	00-608-41
BC9	4/00	Lamb ram	*P. haemolytica*	$9^{\alpha\beta B}$	(β)	wk+	3.5	00-608-52
BC9	4/00	Lam ram	*P. trehalosi*	2	(β)	+	43	00-608-51

Table 2.1 cont.

Animal ID	Date collected mo/yr	Sheep Age Sex	*Pasteurella* isolated	Biovariant	Hemolysis	*lktA* (PCR)[1]	%Toxicity	Isolate #
BC10	4/00	1.5 ram	*P. trehalosi*	2	(β)	+	NT	00-608-61
BC11	4/00	Lamb ewe	*P. haemolytica*	$1^{\alpha B}$	(β)	+	35	00-608-71
BC11	4/00		*P. haemolytica*	$3^{\alpha ES}$	(nh)	wk+	13	00-608-72
BC11	4/00		*P. haemolytica*	$3^{\alpha BE}$	(nh)	+	89	00-608-161
BC11	4/00		*P. haemolytica*	3^{BEX}	(nh)	wk+	33	00-608-164
BC11	4/00		*P. haemolytica*	$16^{\alpha B}$	(nh)	-	NT	00-608-74
BC12	4/00	Adult ewe	*P. trehalosi*	2	(β)	+	NT	00-608-81
BCO1	2/89	Lamb ram	*P. trehalosi*	2	(β)	+	18	89-269
BCO3	9/89	Lamb	*P. trehalosi*	2	(β)	+	76	89-1245-251
BCO4	10/89	No Data	*P. trehalosi*	2	(β)	+	82	89-1634-092
BCO5	1/90	No Data	*P. trehalosi*	2^{C}	(nh)	-	NT	90-107-247
BCO6	4/90	Adult ewe	*P. haemolytica*	3	(β)	NT	NT	90-686-232
BCO7	4/90	Lamb	*P. trehalosi*	3	(β)	+	11	90-850-17
BCO7	4/90		*P. haemolytica*	$10^{\alpha B}$	(β)	+	17	90-850-23
BCO7	4/90		*P. trehalosi*	2	(β)	+	83	90-850-19
SP2	9/97	Adult ram	*P. multocida*	(*gallicida*)	(nh)	NT	NT	97-1079-13
SP2	9/97		*P. haemolytica*	7^{BX}	(nh)	-	NT	97-1079-15
SP4	9/97	Adult ram	*P. haemolytica*	7^{BX}	(nh)	NT	NT	97-1087-04
SP4	9/97		*P. haemolytica*	$16^{\alpha BE}$	(nh)	wk+	27	97-1087-16
SP5	9/98	Adult ram	*P. trehalosi*	4	(β)	wk+	0	98-1242-4
SP5	9/98		*P. trehalosi*	2	(β)	wk+	6	98-1242-1
SP7	9/98	Adult ram	*P. haemolytica*	$16^{\alpha BE}$	(nh)	wk +	NT	98-1242-7
SP7	9/98	Adult ram	*P. trehalosi*	4H	(nh)	-	NT	98-1242-10
SP8	3/99	Adult ram	*P. trehalosi*	2	(nh)	-	NT	99-200-2
SP9	9/99	Adult ram	*P. haemolytica*	7^{BX}	(nh)	-	NT	99-1375-1

Table 2.1 cont.

Animal ID	Date collected mo/yr	Sheep Age /Sex	*Pasteurella* isolated	Biovariant	Hemolysis	lktA (PCR)[1]	%Toxicity	Isolate #
SP9	9/99		*P. trehalosi*	2	(nh)	wk+	0	99-1375-4
SP10	9/99	Adult ram	*P. haemolytica*	$U^{\beta B}$	(nh)	-	NT	99-1354-4
SP10	9/99		*P. trehalosi*	4^{S}	(nh)	NT	NT	99-1354-1
SP11	9/99	Adult ram	*P. haemolytica*	$10^{\alpha B}$	(nh)	+	16	99-1354-12
SP11	9/99		*P. trehalosi*	2^{S}	(nh)	NT	NT	99-1354-11
SP12	9/99	Adult ram	*P. trehalosi*	2^{BS}	(nh)	NT	NT	99-1354-22
SP12	9/99		*P. trehalosi*	2^{B}	(nh)	NT	NT	99-1354-21
SP13	9/99	Adult ram	*P. haemolytica*	$10^{\alpha CG}$	(nh)	-	NT	99-1354-35
SP13	9/99		*P. trehalosi*	2^{S}	(nh)	NT	NT	99-1354-36
SP13	9/99		*P. trehalosi*	4^{S}	(nh)	wk+	0	99-1354-33
SP14	9/99	Adult ram	*P. haemolytica*	$10^{\alpha BC}$	(β)	+	0	99-1360-6
SP14	9/99		*P. trehalosi*	2^{S}	(nh)	-	7.5	99-1360-3

[1] Gene presence determined by PCR
[2] Four years or older.
[3] Beta-hemolytic
[4] Non-hemolytic
[5] Not Tested

Table 2.2 Mean serum titers (log_2) for bovine viral diarrhea (BVD), infectious bovine rhinothracheitis (IBR), parainfluenza-3 (PI_3), and bovine respiratory syncytial virus (BRSV) from bighorn sheep sampled in 1988-1990 (BCO), Big Creek, Idaho (BC) and Spanish Peaks, Montana (SP).

Location	BVD	IBR	PI3	BRSV
BCO	1.0	0.4	1.0	1.4[a]
BC	0.2	0.2	0.4	0.2
SP	0.0	0.0	0.2	0.0

[a] The BCO group had a significantly higher titer (P=0.06) than the BC or SP. No other statistically significant differences existed.

Table 2.3 Blood chemistry values from Big Creek (BC), Big Creek die-off (BCO), and Spanish Peaks (SP) bighorn sheep.

Parameters	BC[af]	Mean	BCO[bf]	Mean	SP[cg]	Mean	Domestic Normals
BUN (mg/dl)	15-33	26	17-25	19.2	8-27[d]	18.1	8.0-20
Creatinine (mg/dl)	1.3-2.1	1.5	1.5-2.3	1.8	0.3-2.8	0.87	1.2-1.9
Na (meq/L)	141-155	148	134-154	147	134-224	163	145-160
K (meq/L)	3.7-8.6	4.9	2.0-7.7	4.4	4.8-8.4[e]	6.6	4.9-5.9
Cl (meq/L)	98-110	104	105-109	107	95-192	132	98-110
Ca (mg/dl)	8.4-10.1	9.4	8.9-10.4	9.4	5.9-11.5	7.7	11.5-13.0
P (mg/dl)	3.1-9.0	6.2	2.1-5.9	4.1	3.1-12.2	5.9	4.0-7.0
Se (ug/g)	0.011-0.50	0.045	0.01-0.05	0.04	--	--	0.05-0.12

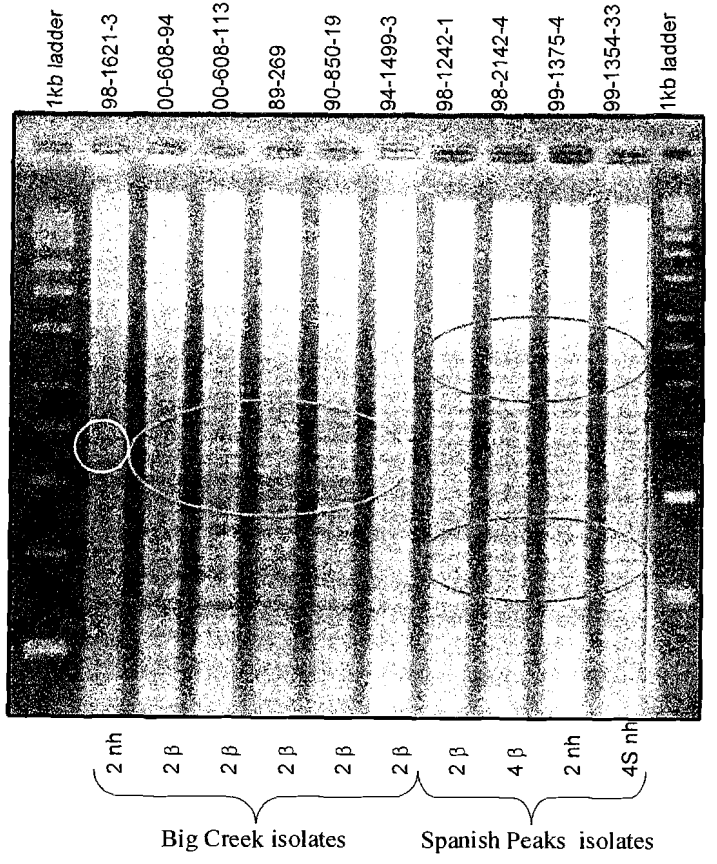
a N=14
b N=5
c N=9
d N=8
[e] N=7
[f] Blood samples
[g] Vitreous humor samples

Table 2.4 Comparison of forage quality from the Big Creek and Spanish Peaks bighorn sheep habitats.

	Spanish Peaks				Big Creek				
Forage Chemistry Values (Dry Matter Basis)	DIET		RANDOM SAMPLE		DIET		RANDOM SAMPLE		REQ[1]
	Mean	(Range)	Mean	(Range)	Mean	(Range)	Mean	(Range)	
% Nitrogen	1.76	(0.6-2.72)	1.53	(0.97-2.02)	1.74	(1.14-2.14)	1.64	(1.22-1.91)	1.50
% Protein	11.0	(5.6-17)	9.53	(6.07-12.64)	10.86	(7.11-13.37)	10.25	(7.6-11.91)	9.4
% ADF[2]	37.37	(24.8-48.7)	43.43	(28.9-53.9)	37.72	(31.3-45.5)	41.12	(34.9-47.1)	**
% Calcium	0.81	(0.4-1.7)	0.87	(0.61-1.09)	0.75	(0.5-1.3)	0.72	(0.58-0.9)	0.20
% Phosphorus	0.18	(0.12-0.3)	0.17	(0.12-0.27)	0.25[a]	(0.16-0.31)	0.25	(0.13-0.31)	0.20
%Magnesium	0.22	(0.11-0.51)	0.20	(0.12-0.34)	0.19	(0.11-0.35)	0.16	(0.09-0.23)	0.12-.18
Zinc (ppm)	27	(22-33)	40	(32-43)	30	(23-41)	32	(26-40)	20-33
Manganese (ppm)	98	(27-141)	136	(76-214)	91	(40-165)	91	(30-216)	20-40
Copper (ppm)	8.5	(4-12)	11	(6-23)	7.8	(7-8)	8.3	(7-10)	7-11
Iron (ppm)	470	(106-1257)	1470	(143-4111)	561	(78-1229)	1538	(100-3252)	30-50
Selenium (ppm)	0.035[b]	(0.03-0.04)	0.043	(0.024-0.052)	0.027	(0.012-0.05)	0.024	(0.02-0.028)	0.1-0.2
TDN[3] (%)	59.94	(46.98-74.33)	53.03	(41.09-69.56)	59.41	(50.71-66.88)	55.66	(48.84-62.8)	55
ME[4] (Kcal/Lb)	984	(771-1220)	871	(674-1142)	977	(832-1098)	914	(802-1031)	909

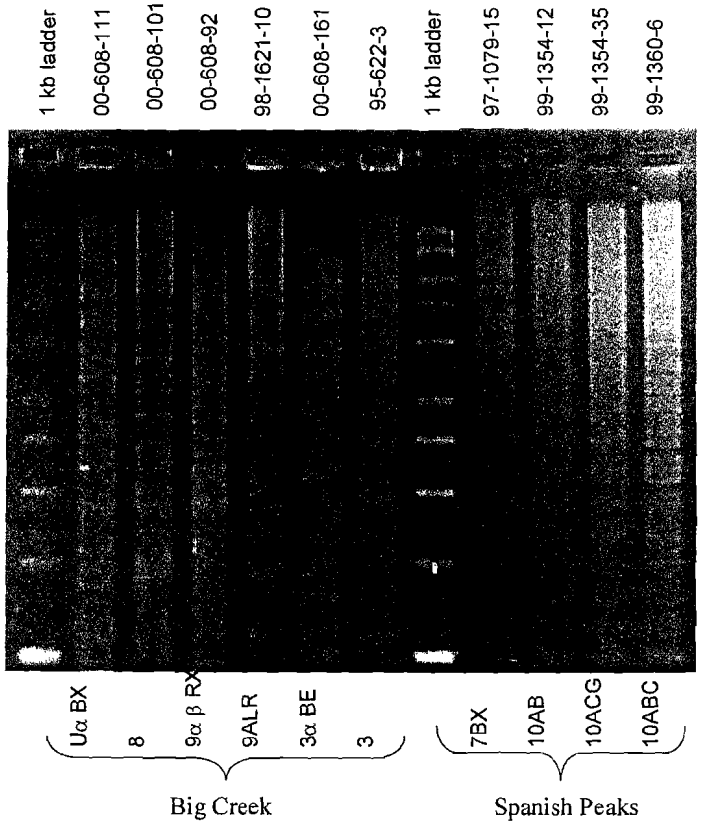
[1] Requirements based on domestic sheep values (NRC, 1985)
[2] Acid Detergent Fiber
[3] Total Digestible Nutrients
[4] Metabolizable Energy
** Variable Data
[a] Significantly higher value of P for BC group (P = 0.08)
[b] Significantly higher value of Se for SP group (P = 0.06)

FIG 2.1 Restriction endonuclease fragment patterns of Big Creek and Spanish Peaks bighorn sheep *Pasteurella trehalosi* isolates cut with HaeIII.



Key to Figure: Nh = non β-hemolytic biovariants
β = β-hemolytic biovariants
Each lane is an isolate number with biovariant and herd designation

Fig. 2.2 Restriction endonuclease fragment patterns of Big Creek and Spanish Peaks bighorn sheep *Pasteurella haemolytica* isolates cut with HaeIII.



Key to Figure: Each lane is an isolate number with biovariant designated at bottom by herd in the Jaworski, et al.(1997) system.

Key to Figure 2.3:

❖ Idaho BC herd isolates (1997-2000)
♦ Idaho BCO herd isolates (1988-1990)
✵ Montana SP herd isolate (1997-2000)
Scale represents from left, 100% correlation to 10% correlation of isolates.

Fig 2.3 Genetic relationships of *Pasteurella/Mannheimia haemolytica* and *Pasteurella trehalosi* from Big Creek, Idaho and Spanish Peaks, Montana bighorn sheep herds.

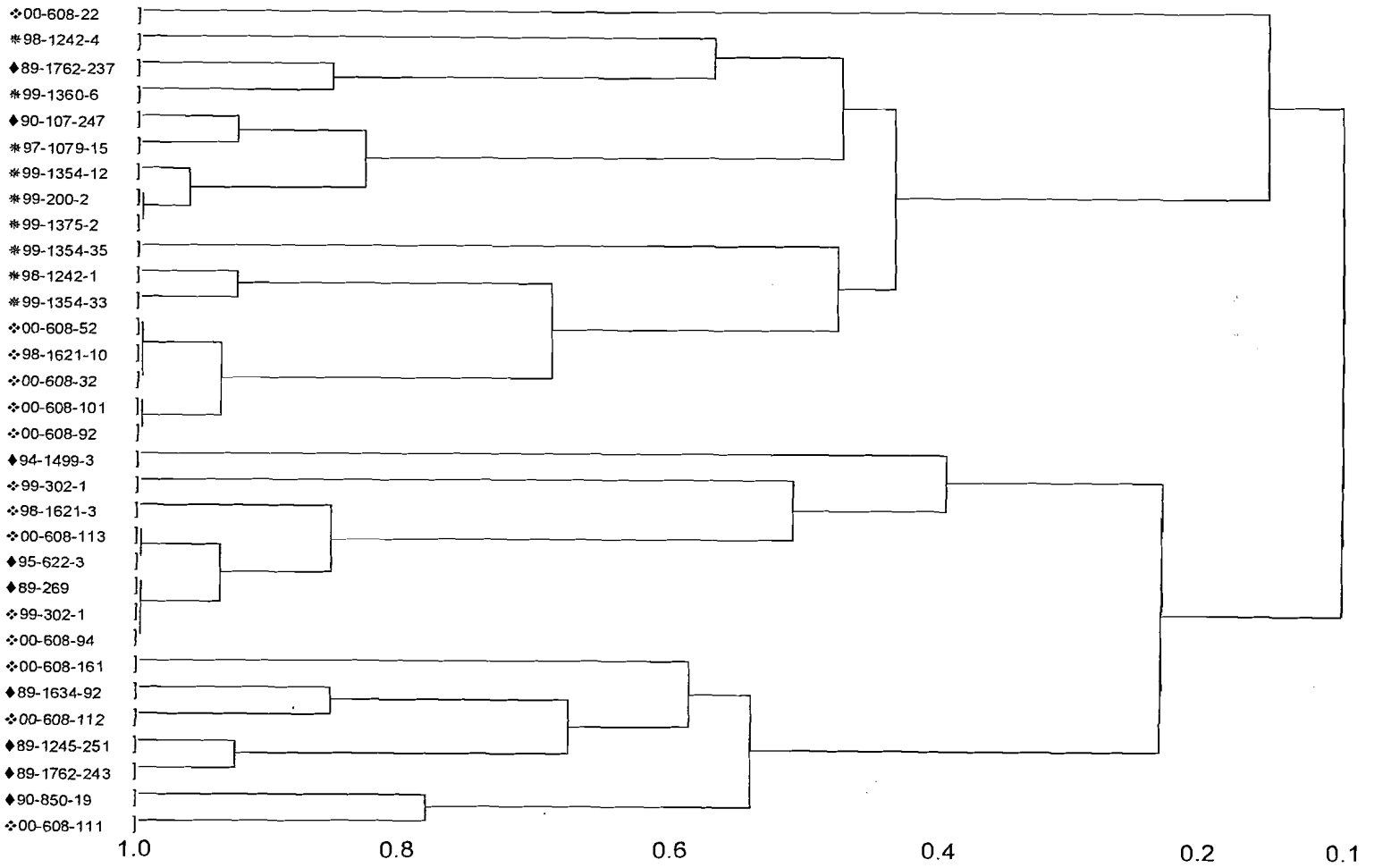
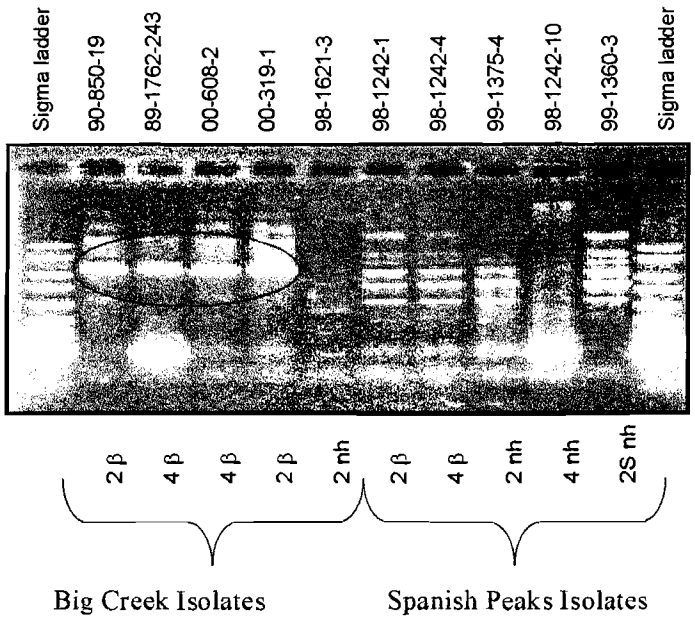
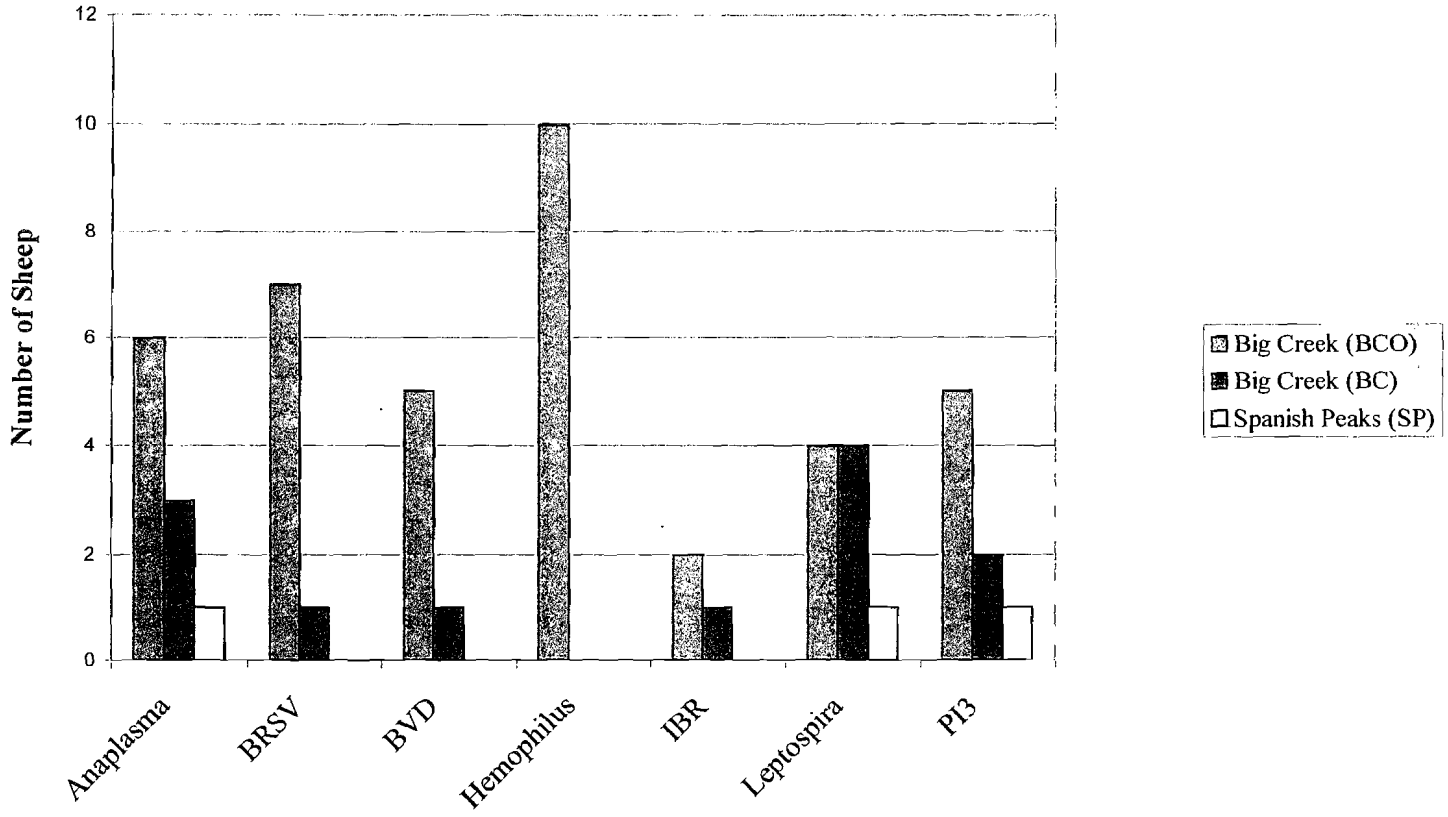


Fig. 2.4 PCR of leukotoxin gene from *Pasteurella trehalosi* isolates of bighorn sheep in Big Creek, Idaho and Spanish Peaks, Montana.



Key to figure: Nh = non β-hemolytic biovariants; β = β-hemolytic biovariants
Each lane represents on isolate, indicated by number.
Circle representing 1145bp bands in multiple isolates from BC bighorn sheep

Fig. 2.5 Serologic Titres to Selected Diseases for the Big Creek and Spanish Peaks Bighorn Sheep Herds



Key to figure: Total number of sheep with any level of titer.

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APPENDICES

APPENDIX A

Study Area Plant Species List

Table 2.5 Plant species list for Big Creek (BC) and Spanish Peaks (SP) bighorn sheep forage.

BC	
Scientific Name	Common Name
Achillea millifolium	western yarrow
Agropyron spicatum	bluebunch wheatgrass
Bromus tectorum	cheatgrass brome
Carex spp.	sedges
Cercocarpus intercedens	mountain mahogany
Cirsium hookeri	thistle
Deschampsia caespitosa	tufted hairgrass
Eriogonum strictum	wild buckwheat
Festuca idahoensis	Idaho fescue
Galium boreale	catchweed bedstraw
Juncus spp.	rushes
Lycopodium spp.	moss
Mitella spp.	mitella
Poa secunda	Sandberg bluegrass
Poa pratensis	Kentucky bluegrass
Tragopogon dubius	salsify
Verbascum thapsus	mullien

Table 2.5 cont.

SP	
Scientific name	Common name
Achillea millefolium	western yarrow
Arctostaphylos uva-ursi	kinnickinnick
Agropyron intermedium	intermediate wheatgrass
Agropyron spicatum	bluebunch wheatgrass
Antennaria lanata	pussytoes
Aquilegia spp.	columbine
Artemesia frigida	fringed sage
A. spinescens	budsage
Berberis repens	Oregon grape
Bromus carinatus	mountain brome
Bromus tectorum	cheatgrass brome
Carex spp.	carex
Cystopteris fragilis	bladder fern
Deschampsia spp.	hairgrass
Eriogonum strictum	wild buckwheat
Festuca idahoensis	Idaho fescue
Festuca scabrella	rough fescue
Geranium viscossissimum	sticky geranium
Geum triflorum	prairie smoke

Table 2.5 cont.

Scientific name	Common name
Gaillardia aristata	blanket flower
Cynoglossum officinale	houndstongue
Lycopodium spp.	moss
Mitella spp.	mitella
Oxyria spp.	mountain sorel
Phacelia hastata	waterleaf
Poa spp.	bluegrass
Rosa woodsii	Wood's rose
Sedum spp.	sedum
Symphoricarpus occidentalis	snowberry
Trifolium spp.	clover

APPENDIX B

Animal Care and Use Committee Approval

University of Idaho
Animal Care and Use Committee

Date: Friday, March 02, 2001
To: Alton C. Ward
From: University of Idaho
Re: Protocol 8047
Evaluation of factors affecting health of bighorn sheep

Your requested renewal of the animal care and use protocol shown above was reviewed by the University of Idaho on Friday, March 02, 2001.

This protocol was originally submitted for review on: Thursday, September 24, 1998
The original approval date for this protocol is: Friday, September 25, 1998
This approval will remain in affect until: Tuesday, September 25, 2001
The protocol may be continued by annual updates until: Tuesday, September 25, 2001

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Brad Williams DVM

IACUC Representative