A CALIFORNIA BIGHORN SHEEP LAMB MORTALITY INVESTIGATION IN AN EAST FRASER RIVER HERD, BC, CANADA

JASON STRUTHERS, Canadian Cooperative Wildlife Health Centre, Department of Veterinary Pathology, 52 Campus Drive, Saskatoon, SK, S7N 5B4, Canada

HELEN SCHWANTJE, Fish, Wildlife and Habitat Management Branch, Ministry of Forests, Lands and Natural Resource Operations, 2975 Jutland Rd, Victoria, BC, V8W 9M8, Canada

DOUG JURY, Ministry of Forests, Lands, and Natural Resource Operations, 1259 Dalhousie Drive, Kamloops, BC, V2C 5Z5, Canada

CHRIS PROCTER, Ministry of Forests, Lands, and Natural Resource Operations, 1259 Dalhousie Drive, Kamloops, BC, V2C 5Z5, Canada

TRENT K. BOLLINGER[1], Canadian Cooperative Wildlife Health Centre, Department of Veterinary Pathology, 52 Campus Drive, Saskatoon, SK, S7N 5B4, Canada

Abstract: Chronic poor recruitment and lamb mortality were investigated in a California bighorn sheep (*Ovis canadensis californiana*) herd from British Columbia's (Canada) interior in the summer of 2011. Daily monitoring of a band of sheep from mid-June to mid-July identified coughing and diarrhea in lambs which increased in prevalence over time until 32–39% of lambs were affected near the end of the study period. Two euthanized sick lambs and one lamb found dead had severe bronchopneumonia. *Mycoplasma ovipneumoniae* was determined to be a significant pathogen in the lung based on characteristic histological lesions and its identification using polymerase chain reaction. Other bacteria isolated from the lungs and the tympanic bullae include: *Bibersteinia trehalosi*, *Pasteurella* spp., *Mannheimia haemolytica*, and *Streptococcus suis*. Although lungworm (*Protostrongylus* spp.) was initially suspected to be a contributing cause of pneumonia, compatible histological lesions were not evident and only one adult nematode was found in lungs at autopsy. Low counts of lungworm larvae in feces of lamb and adult sheep collected during the summer supported this result. Our findings suggest *Mycoplasma ovipneumoniae* was a major cause of morbidity in these lambs and we hypothesize additional factors, such as secondary bacteria, inclement weather, and predation of sick lambs that result in high lamb losses in some years. Further research is required to confirm these findings and to determine the relative importance of additional factors on poor recruitment in this herd.

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Bighorn sheep (*Ovis canadensis)* populations throughout western North America have experienced high losses due to infectious disease (Besser et al. 2008, 2012). California bighorn sheep (*O. c. californiana*) in British Columbia (BC, Canada) are no exception and several herds have experienced population declines thought to be related to disease. The Fraser River Valley metapopulation of California bighorn sheep, which comprises 60% of the total Canadian population of this subspecies, reportedly declined 25% in 1984, and 38% in 1995 (Fraser River California Bighorn Sheep Advisory Committee 2004). More recently, the number of animals of the East Fraser population, a constituent of the Fraser metapopulation, declined from 850 animals in 1993 to 350 in 2007, and is currently estimated at 450; 41% of this increase being a result of a sheep translocation performed in 2009 (C. Procter and D. Jury, Ministry of Forests, Lands, and Natural Resource Operations, unpublished data).

Within this population, it appears that herds east of the Fraser River (Lillooet to Canoe Creek) are recovering, with the exception of a band of bighorn sheep within the Kelly Creek-Canoe Creek (KCCC) herd and a band within the

[1] Email: trent.bollinger@usask.ca

Lillooet-Kelly Creek (LKC) herd. For example, the KCCC band had a lamb:ewe ratio of 11% in August of 2010 and the LKC band had a lamb:ewe ratio of 5% in November of 2009 (C. Procter and D. Jury, unpublished data). These herds were previously identified in a 2004 management plan as being at high risk of population declines due to the presence of multiple threats, including: disease/parasites, predation, livestock disturbance, and forage competition. The report further speculated that persistently low herd numbers were attributable to high lamb mortality in some years (Fraser River California Bighorn Sheep Advisory Committee 2004).

Autopsies performed on two lambs (6 weeks and 8 weeks of age) from the Pavilion band of the LKC herd in 2005 revealed emaciation and polymicrobial bronchopneumonia with pleuritis. Despite numerous tests for known sheep pathogens, a consistent infectious etiology was not identified. At autopsy, pneumonia was found to be the primary cause of death which correlated with annual observations of lambs from both bands showing lethargy, poor body condition, and respiratory distress.

Based on these findings and the localized nature of the poor recruitment, an investigation of the causes of poor lamb recruitment in a band of sheep from the KCCC herd was initiated in the summer of 2011. A 4 week field program was organized to confirm and characterize lamb morbidity, attempt to collect dead lambs for examination, and identify disease-causing agents responsible for poor lamb survival. In accordance with previous autopsy results and lamb morbidity observations, investigators hypothesized that infectious respiratory disease was an important problem, but all causes of morbidity and mortality were investigated.

METHODS

Study Site

The range of the KCCC herd extended from Canoe Creek to Kelly Creek on the east side of the Fraser River. The band under study used the eastern banks of the Fraser River, southwest of Big Bar Mountain, and northeast of the Fraser River Big Bar Ferry crossing, the latter 72 km from Clinton, British Columbia, Canada. This area includes Big Bar Creek and is a constituent of British Columbia's Lillooet land district (approximate UTM coordinates N560689 E5676150). Although some sheep of the KCCC herd may migrate to alpine summer range, the band of this study were considered non-migratory and known to make extensive use of irrigated alfalfa hayfields located at the southern aspect of their range.

The study area spanned approximately 7 km by 1.5 km and was accessed by an all-terrain vehicle trail snaking through its center, or by roads running adjacent to the hayfields.

The altitude ranged from 330 m to 1100 m and the landscape was composed of variable terrain: vertical rock face cliffs, sparsely treed bluffs, sloped outcrops, and deep carved valleys separated by intermittent steppes. Tree density intensified with increasing distance from the Fraser River and at an altitude of 800 m. Irrigated hayfields sloped slightly towards the river at an altitude of 490 m to 580 m and covered a surface area of approximately 1600 m by 300 m. Approximately 3000 m north of the hayfields and at an altitude of 340 m, there is a steppe immediately adjacent to the river which was also used extensively by lambs and ewes.

The study site was within the Central Interior Ecoprovince characterized by cold winters, warm summers, and a precipitation maximum in late spring or early summer (Demarchi 2011). More specifically, the Big Bar band occupied the Fraser River Basin Ecosection. This ecosection has a warm and dry summer climate with minimal moisture, and winters can be cold and bring deep snow. Vegetation reflected these dry conditions, and was predominated by bunchgrasses, including big sagebrush, bluebunch wheatgrass, and needle-and-thread (Demarchi 2011).

Field Observations

Daily observations of lambs and ewes occurred from 21 June until 18 July 2011. During this period, the number of sheep present, lamb:ewe ratios, and age and sex structure (when possible) were recorded; also, sick lambs were identified along with their clinical signs. None of the sheep were individually marked but any significant identifying characteristics were noted.

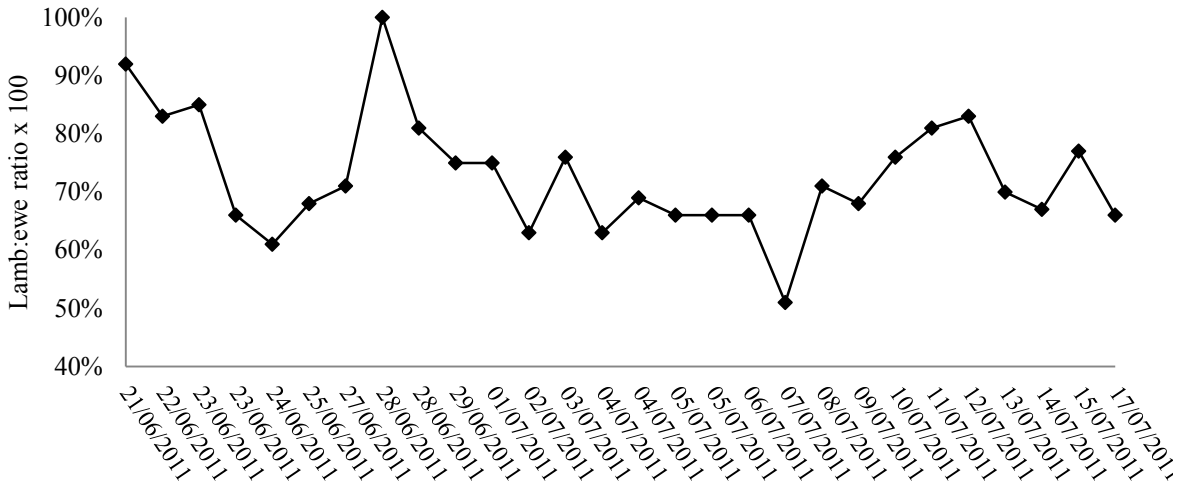

Fig. 1. Ratio of lambs to ewes observed between 21 June and 17 July 2011. Number of ewes observed each day was variable and depended on which groups were seen.

Each day, a concerted effort was made to locate and count a maximum number of ewes and lambs in order to calculate lamb:ewe ratios (Fig. 1). All ewes were counted regardless of their reproductive status; therefore, the presence of non-breeding immature and geriatric ewes could have resulted in low ratios.

On some days, it was necessary to calculate and plot (Fig. 1) two lamb:ewe ratios. This was done in situations where it was not possible, according to time and location, to determine if observed animals had already been previously counted.

Fecal Lungworm Assessment

Throughout the 4 week study period, fresh bighorn sheep feces were opportunistically collected from observed groups while prioritizing lamb feces. Lamb feces were easily differentiated from those of adults by their smaller size. Samples (n = 76) were retrieved within a few hours of defecation, transferred to labeled whirl-pak bags, and chilled prior to processing.

A modified Baermann technique (Forrester and Lankester 1997) was used to quantify lungworm larvae in fecal samples. The number of larvae in the 76 fecal samples is reported in larvae per (dried) gram (LPG) of feces. Initially, at least one larvae per sample was identified microscopically to genus, and later at least one larvae was identified for every ten counted.

Twenty-six parasite samples were transferred to 3 ml microvial tubes, frozen initially at -20º C, and later stored at -80º C. Similarly, unprocessed fecal samples were frozen under the same conditions in their labeled bags.

Four fecal samples, which were diarrheic (BB82, BB89, BB90, and BB91), were immediately frozen. Lambs were observed to be scouring prior to sample collection, and the small piles of diarrheic feces recovered indicated that at least three of the four samples were from lambs.

Post-mortem Evaluation and Diagnostic Tests

On 15 July and 17 July 2011, two ill lambs (lambs 1 and 2) in severe respiratory distress were killed by gunshot to the neck, severing the spinal cord. Autopsies were performed immediately. Tissues collected for histopathology were placed in 10% neutral buffered formalin and selected duplicates were frozen. On 19 July 2011 a dead lamb (lamb 3) was retrieved from the hayfield by the landowner. The carcass was frozen and shipped to the Canadian Cooperative Wildlife Health Centre in Saskatoon, Saskatchewan for complete autopsy. In the three lambs, sections from all lung lobes were sampled for histopathology.

Fresh or frozen sections of lung from all three lambs were submitted to Prairie Diagnostic Services Inc. (PDS, Saskatoon, Saskatchewan, Canada), where they were cultured aerobically at 37º C on blood, MacConkey, and chocolate agar media. Swabs of the tympanic bullae, pharynx,

and affected lung surface from lambs 1 and 2 were collected using sterile polyester tipped applicators (Puritan Medical, Guilford, Maine, USA) and shipped to the laboratory on Leighton transport media. Swabs from lamb 1 were similarly cultured at the Animal Health Center (AHC, Abbotsford, BC, Canada) and those of lamb 2 cultured at PDS. Swabs and lung tissue from lamb 2 were also submitted for culture on Hayflick's broth and agar.

Frozen lung from each lamb was tested for infectious bovine rhinotracheitis (IBR), parainfluenza virus type 3 (PI3), respiratory syncytial virus (RSV), and bovine viral diarrhea (BVD) at PDS using immunofluorescence. In all cases, frozen lung tissue was cut onto chrome alum coated slides, dried, fixed in acetone, and then incubated with primary monoclonal antibodies. Monoclonal antibodies used were clones 3F11 and 1H6 (Dr. V. Misra, University of Saskatchewan, Saskatoon, SK) for IBR; clone 2E2 (Dr. D. Haines, University of Saskatchewan, Saskatoon, SK) for PI3; clone 8G12 (Dr. G. Anderson, University of Nebraska, Lincoln, NE) for RSV; and clones 20.10.6 and 15.C.5 (Dr. E. Dubovi, Cornell University, Ithaca, NY and IDEXX laboratories) for BVD. Following a rinse cycle, slides were incubated with the fluorescein isothiocyanate (FITC)-conjugated secondary antibody (goat anti-mouse IgG FITC conjugate (Cappel)) and binding was detected using a fluorescent microscope.

Nested-polymerase chain reaction (PCR), based on the amplification of part of the 16S rRNA, was used to detect *Mycoplasma* spp. Primary (GPO1 and MSGO) and secondary (Myins and MSGO) genus-specific primers were used as described by Yoshida et al. (2002). DNA was extracted from diseased lung using a DNeasy Blood & Tissue kit (Qiagen Inc., Toronto, Ontario, Canada). Two PCR reactions were run for each sample; 2 ul of DNA was combined with 48ul of a master mix preparation that contained 4.0 ul of primary or secondary primers. Both mixtures were put through a thermal cycler. Following a standard protocol, speciation of *Mycoplasma* spp. was done by sequencing the PCR amplicons with an applied Biosystem's Gene Amplification PCR system 9700 (National Research Council, Saskatoon, Saskatchewan, Canada). Obtained sequences were compared to those archived in GenBank ® (National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD, USA). A similar nested-PCR technique was performed on lung for herpesvirus detection. For each tissue submission, 5 ul of DNA was combined with 45 ul of a master mix preparation that contained 7.5 ul (DFA, ILK, KGI) and 5.0u l (TGV, IYG) of primers, during primary and secondary PCR, respectively; both products were put through a thermal cycler (VanDevanter et al. 1996). PCR amplicons were visualized using a QIAxel DNA screening kit (Qiagen Inc., Toronto, Ontario, Canada).

Formalin-fixed tissues were processed routinely for histology; embedded in paraffin wax, sectioned at 4 μm, stained with hematoxylin and eosin, and examined microscopically.

RESULTS

Field Observations

Clinical signs displayed by sick lambs included coughing, diarrhea, poor body condition, and lethargy. Coughing was infrequent, episodic, and of variable duration and severity. Occasionally, bouts of coughing were severely debilitating and lasted up to 1 minute, with violent head jerking and clearly audible dry coughs; eventually the lamb collapsed. A second common clinical sign was diarrhea; lambs had darkly soiled rumps, thighs, and hocks with encrusted fecal material matted in their hair. A few lambs were thin as evidenced by ribs, verterbral spines and other bony protuberances being prominent. Some lambs were considered weak or lethargic, since they moved slowly and would frequently lie down, often at inappropriate times (e.g. during group movements). Lambs considered sick had scruffy hair coats. At least one was lame but otherwise appeared healthy, suggesting a traumatic etiology. Most sick lambs had several of these clinical signs.

Coughing and diarrhea increased in prevalence and severity throughout the study; although, on 21 June 2011, during an initial visit to the site, two severely sick lambs with respiratory disease were observed and were suspected to have died within the next 48 hours. Their carcasses were never found. From 22 June until 29 June 2011 only two more coughing lambs were seen in 154 lamb observations. Comparatively, on 15 July 2011, an observation of 31 lambs identified 32–39% of

lambs showing one or more of the aforementioned clinical signs.

Lamb:ewe ratios were calculated from daily group observations within the study area. A concerted effort was made to count all of the ewes and lambs each day; however, this was limited by the terrain and the frequent movement and mixing of groups. As a result, ratios varied depending on which groups were observed that day. In this investigation lamb:ewe ratios were used to track trends in lamb mortality, especially if carcasses or mortality was not directly observed. After the initial loss of two lambs, lamb:ewe ratios remained relatively stable at around 70% (Fig. 1) indicating little or no lamb mortality.

Fecal Lungworm Assessment

Seventy-six (70 ewe and 6 lamb) fecal samples were collected. Larvae per gram ranged from 0–51, and the average LPG in ewe and lamb feces was 6 and 1, respectively.

Post-mortem Evaluation and Ancillary Diagnostic Tests

Pathology was similar in all lambs, only varying in severity. Autopsied lambs had moderate to marked diarrhea, characterized by matting of hair coats by feces from the perianal area to the hocks. They were thin; subcutaneous and abdominal fat was absent and pericardial fat was minimal. Bilaterally, 20–80% of the anteroventral lung was firm, consolidated, dark red to tan, and had a lobular pattern. In lambs 1 and 2, airways were partially occluded by a viscous, mucopurulent exudate. Freezing artefact in lamb 3 complicated the interpretation of airway content. Despite meticulous dissection of airways, only one *Protostrongylus rushi* pulmonary nematode was identified (from lamb 1). A friable adhesion of the cranial right lung lobe to the pericardium was observed in lamb 3. In all three lambs, mediastinal and bronchial lymph nodes were enlarged and had variably reddened cortices, and in lamb 3, the retropharyngeal lymph node was similarly enlarged. The gastrointestinal tract of all lambs was unremarkable.

Significant microscopic findings were limited to the lungs in all three lambs. Lesions consisted of atelectasis, bronchial epithelium hyperplasia, lymphoplasmacytic hyperplasia and cuffing of airways, mild and multifocal thickening of alveolar septa by lymphocytes and plasma cells, and a marked increase in intra-alveolar macrophages. The airway lumens of lambs 1 and 2 were often narrowed, contained minimal to moderate amounts of neutrophils and sloughed epithelium, and were usually surrounded by an intact bronchial/bronchiolar ciliated epithelium. Comparatively, airways and less frequently, alveoli of lamb 3 were diffusely and markedly expanded by neutrophils that effaced airway epithelium; these foci were multifocally admixed with bacterial colonies. Cross-sections of nematode profiles were absent from examined lung sections.

Accordingly, a lymphoplasmacytic to minimally suppurative bronchopneumonia with prominent airway cuffing and regional lymphadenopathy was identified in lambs 1 and 2. Lamb 3 had a moderate suppurative bronchopneumonia with prominent lymphoplasmacytic airway cuffing and intralesional bacteria, with regional lymphadenopathy and locally extensive fibrinous pleuritis.

Tests for BVD, herpesvirus, RSV, and PI3 were negative in all lambs. Bacterial culture and polymerase chain reaction results are detailed in Table 1. PCR consistently detected *Mycoplasma ovipneumoniae* in affected lung tissue collected from each lamb. Generally, bacterial culture results were mixed and dissimilar among lambs, and growth intensity varied from low (lamb 1 and 2) to high (lamb 3).

DISCUSSION

In recent years it appears that collectively the KCCC herd's population has increased, but remains low relative to surveys in the late 1980s and early 1990s. Surveys conducted in April 2011 observed 247 sheep (C. Procter, unpublished data), an increase relative to surveys done in 2006 which observed 151 sheep (23 lambs, 98 ewes, 30 rams), yet well below results from a survey in 1990 when 525 bighorn sheep were observed (Lemke and Jury 2006).

The population's decline from 1990 onward is attributed to disease and subsequent management practices. In the autumn of 1993 weak lambs and poor lamb survival was reported, and in 1995,

Table 1. Bacteriology results (culture and nested-polymerase chain reaction (PCR)) from three autopsied lambs.

Lamb/ Lab[b]	Lung culture			PCR			Swab culture[a]				
	1	2	3	1	2	3	1			2	
							L	P	T	L	T
PDS	*B. trehalosi* 2+ Few *Pseudomonas*	*Pasteurella* spp. 1+ *Pseudomonas* spp. 1+	*S. suis* 4+ *P. multocida* 1+	*M. ovipneumoniae* (99% identity)			ND			-	A
AHC	*P. multocida* 1+	ND	ND	ND			-	ND	B	ND	ND

M. ovipneumoniae = Mycoplasma ovipneumoniae; S. suis = Streptococcus suis; B. trehalosi = Bibersteinia trehalosi
[a] = L is lung surface; P is pharynx; T is tympanic bullae
[b] Lab = Diagnostic laboratory (PDS: Prairie Diagnostic Services or AHC: Animal Health Center)
A = 1+ *Lactobacillus* spp.; 1+ *Mannheimia haemolytica*
B = 1+ *P. multocida*; few *E.coli* (non-haemolytic)
- = negative (no growth)
ND = not done

lungworm and polymicrobial pneumonia was confirmed as the cause of mortality in a lamb. In an effort to mitigate this decline 102 animals were transplanted from the KCCC herd to the western USA between 1994 and 1996, and ewe harvests were increased under Limited Entry Hunting. Despite practices to favor lamb growth and recruitment, which initially may have worked, from 1999 to 2006 the spring lamb:ewe ratio declined from 39% to 23% (Lemke and Jury 2006).

Currently, although most bands of the KCCC herd appear to be doing well, recruitment rates in the Big Bar area have been consistently low, and the result has been a stagnant population (C. Procter, unpublished data). Midway through our field program, a maximum of 96 sheep (32 lambs, 46 ewes, 18 rams) were observed at one time.

The number of lambs that died during the 2011 study period was lower than anticipated based on past survey observations by regional wildlife biologists. In 2011, only three lambs are known to have died, one of which was found dead, while two others were euthanized on account of severe illness. These latter would have likely died naturally, but we cannot say for certain.

Clinical disease was observed in lambs estimated between 50 and 90 days of age. Familiarity with the band and herd predicts the peak of lambing during late April (C. Procter, personal observation), thus sick lambs observed on July 15 and the dead lamb recovered on July 19 would have been approximately 76 and 80 days old, respectively.

In spite of clinical disease in approximately 1/3 of the lambs, the lamb:ewe ratio remained relatively stable throughout the summer. Mortality did occur later in the year; aerial surveys, conducted in March 2012, revealed a lamb:ewe ratio of a mere 8% (50–52 ewes, 2–4 lambs; C. Procter, unpublished data). When and why these lamb losses occurred is unknown, but a decline of this severity is not likely due entirely to predation, but rather disease may be a contributing factor. Especially since adjacent bands of sheep, which should be exposed to comparable predation, appear to have higher recruitment rates.

Although the lamb sample size was low and findings are only from one year, the results of this investigation allowed significant preliminary conclusions to be drawn.

Observations of sick lambs confirmed previous reports of respiratory disease, but also identified concurrent diarrhea for the first time. Post-mortem examination and histology of the gastrointestinal tract failed to reveal the cause. Moreover, feces and segments of intestine from autopsied lambs were tested for parasites and bacteria, yet no significant pathogens were identified. Future studies will focus on obtaining better samples for diagnostic investigation. Additionally, the impact of an alfalfa-rich diet has been postulated.

The role of certain respiratory pathogens in respiratory disease in the Big Bar lambs is becoming clearer. Fecal analysis concomitant with little sign of pulmonary nematodes, and an absence of compatible gross and histologic lesions in autopsied lambs, indicates lungworm is not

important in young lambs in this band. Furthermore, many respiratory viruses were not detected. Rather, the distribution and gross appearance of pulmonary lesions suggested a bacterial cause. Histology further confirmed this, and the presence of marked lymphoplasmacytic cuffing of airways, a lesion regarded to be nearly pathognomonic for *M*. *ovipneumoniae* infection in domestic sheep (Nicholas et al. 2008), prompted pursuit of *Mycoplasma* spp. using PCR. Although an array of bacteria were isolated (Table 1), these were both inconsistent in type and number among lambs. Interestingly, as detailed in Table 1, lamb 1 and lamb 2 had relatively low bacterial growth in comparison to lamb 3. The difference may be attributed to the stage or chronicity of pulmonary disease; the two first lambs were euthanized by gunshot early in the pathogenesis of pneumonia, relative to lamb 3, which died naturally of bacterial respiratory disease. Additional findings of fibrinous pleuritis, neutrophil-rich bronchopneumonia, and numerous bacterial colonies in lamb 3 support the culture results and indicate a more severe and advanced pneumonia.

The fact that *Mycoplasma* spp. was not isolated on aerobic culture is not surprising, considering the organism is fastidious and requires particular media nutrients and certain oxygen levels to thrive (Nicholas et al. 2008). Polymerase chain reaction did succeed in consistently identifying, with a 99% similarity, *M*. *ovipneumoniae* in the diseased pulmonary tissues of all three lambs.

Therefore, gross findings, microscopic lesions, and results of ancillary testing confirm the importance of bacterial pneumonia in these lambs. Molecular techniques consistently identified *Mycoplasma ovipneumoniae* in diseased lung, at early and late stages of respiratory disease, supporting the pathogen's primary role in bronchopneumonia in lambs of this band of the KCCC herd during the summer of 2011. These results are in agreement with recent research by others (Besser et al. 2012), which indicates that *Mycoplasma ovipneumoniae* can act as a primary pathogen to predispose bighorns to secondary microbial invasion. This may lead to fatal suppurative polymicrobial bronchopneumonia, such as with lamb 3. The virulence determinates of *Mycoplasma* spp., such as its polysaccharide capsule, its ability to evade the host's humoral response, and its detrimental effect on respiratory cilia compromise the lung's protective mechanisms and allow colonization by opportunistic flora (Nicholas et al. 2008; Ongor et al. 2011).

The preliminary conclusions of our investigation of poor lamb recruitment in the Big Bar band of the KCCC herd indicate infectious disease, and especially pneumonia, is a significant cause of morbidity and mortality in lambs. We also conclude that *M*. *ovipneumoniae* was the initiating cause of lamb pneumonia, and was involved in potentially fatal bacterial bronchopneumonia. Further study is required to determine if this is a consistent finding among years, the relative importance of other pathogens in causing mortality, better understanding the causes of diarrhea, and how factors such as: inclement weather, the concentration of lambs and ewes on hayfields, contact with domestic animals, and predation, etc. contribute to mortality.

This work identified *M*. *ovipneumoniae* as a likely candidate for initiating pneumonia in bighorn lambs and, if this is a consistent finding, will provide a focus for future research which may lead to development of targeted, novel, mitigation strategies.

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