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Source: Journal of Wildlife Diseases, 53(1) : 126-130

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/2016-05-097>

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## How Respiratory Pathogens Contribute to Lamb Mortality in a Poorly Performing Bighorn Sheep (*Ovis canadensis*) Herd

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**ABSTRACT:** We evaluated bighorn sheep (*Ovis canadensis*) ewes and their lambs in captivity to examine the sources and roles of respiratory pathogens causing lamb mortality in a poorly performing herd. After seven consecutive years of observed December recruitments of <10%, 13 adult female bighorn sheep from the remnant Gribbles Park herd in Colorado, US were captured and transported to the Thorne-Williams Wildlife Research Center in Wyoming in March 2013. Ewes were sampled repeatedly over 16 mo. In April 2014, ewes were separated into individual pens prior to lambing. Upon death, lambs were necropsied and tested for respiratory pathogens. Six lambs developed clinical respiratory disease and one lamb was abandoned. Pathology from an additional six lambs born in 2013 was also evaluated. *Mycoplasma ovipneumoniae*, leukotoxigenic *Mannheimia* spp., leukotoxigenic *Bibersteinia trehalosi*, and *Pasteurella multocida* all contributed to lamb pneumonia. Histopathology suggested a continuum of disease, with lesions typical of pasteurellosis predominating in younger lambs and lesions typical of mycoplasmosis predominating in older lambs. Mixed pathology was observed in lambs dying between these timeframes. We suspected that all the ewes in our study were persistently infected and chronically shedding the bacteria that contributed to summer lamb mortality.

**Key words:** *Bibersteinia trehalosi*, Bighorn sheep, *Mannheimia*, *Mycoplasma ovipneumoniae*, *Ovis canadensis*, *Pasteurella multocida*, pneumonia, respiratory disease.

Respiratory disease is a major limitation to recovery and management of bighorn sheep (*Ovis canadensis*) populations. While pneumonia outbreaks can result in catastrophic losses in adult bighorn sheep populations (Cassaigne et al. 2010), the more significant concern is the sustained effect on lamb survival. Lamb survival and recruitment can

remain depressed for years to decades after a respiratory disease outbreak in a bighorn sheep population (George et al. 2009; Cassirer et al. 2013). This can lead to poorly performing herds of chronically infected, aging adults.

The Gribbles Park (also known as Badger Creek) bighorn sheep herd was transplanted into Gribbles Park, Colorado, US (38°38'34"N, 105°47'34"W), in 1990 (George et al. 2009). Initially the herd grew, but by the early 2000s lamb recruitment and numbers declined. Respiratory pathogens including leukotoxigenic (lkt+) *Mannheimia glucosida*, lkt+ *Bibersteinia trehalosi*, *Mycoplasma ovipneumoniae*, *Pasteurella multocida*, and sinus tumors were present (Sirochman et al. 2012; Miller et al. 2013). Adult mortality was not documented, although wildlife managers noted a decline in adult numbers beginning around 2001. During 2004–10, management actions taken to improve herd health included winter feeding, mineral supplementation, antibiotics, deworming, vaccination, and hyperimmune serum administration (Sirochman et al. 2012). There was no response to these interventions and recruitment dropped to zero in 2007. With effectively no recruitment and minimal immigration, the herd was deemed unviable. In 2013, Colorado Division of Parks and Wildlife removed the remaining 13 ewes for experimental use and the single remaining ram disappeared after removal of the ewes.

Eleven Gribbles Park ewes entered the Thorne-Williams Wildlife Research Center in Wyoming in March–April 2013 after natural breeding in the field; two others, initially held in isolation, were added in October 2013. During April–August 2013, eight ewes were

involved in a study evaluating efficacy of the antibiotic tildipirosin (Zuprevo, Merck Animal Health, Madison, New Jersey, USA) to decrease bacterial pathogen transmission to lambs (Raghavan et al. 2016). Of the seven lambs born in 2013, one was abandoned and six succumbed to bronchopneumonia. A Wyoming ram carrying similar bacterial pathogens was introduced for breeding in November 2014.

All adult ewes were sampled serially over 16 mo to evaluate pathogen occurrence (see Supplementary Material Table S1). Samples taken included nasal swabs, tonsil swabs, and venous blood. Nasal swabs were tested for *M. ovipneumoniae* through culture (see Supplementary Material, in Diagnostic Methods section; Jennings-Gaines 2016) and PCR assay (McAuliffe et al. 2003). Tonsil swabs were tested for *Pasteurellaceae* by culture (see Supplementary Material Diagnostic Methods) and PCR. For *Pasteurellaceae* PCR, each sample was screened for the leukotoxin A (*lktA*) gene of *Pasteurellaceae* that includes *Mannheimia* species and *B. trehalosi* (Davies et al. 2001). Positive samples were then analyzed by PCR for the *lktA* gene of *Mannheimia* species (*Mannheimia hemolytica*, *Mannheimia glucosida*, and *Mannheimia ruminalis*; Shanthalingam et al. 2014). Samples positive on initial *lktA* PCR but negative on the second test were categorized as *lktA*-positive *B. trehalosi*. We further screened samples positive for *Mannheimia lktA* with a third PCR specific for the *lktA* gene of *M. haemolytica* or *M. glucosida* (Angen et al. 2009).

To evaluate the role of each ewe as the sole source of infection to her lamb, each pregnant, surviving ewe ( $n=7$ ; four ewes had died from chronic illness in the preceding year and two were not pregnant) was placed in an individual pen on 29 April 2014 (2–6 wk prior to lambing) such that contact was limited to respective ewe-lamb pairs during the neonatal period. Lambs were scored daily for clinical signs of respiratory disease (none, mild, moderate, severe). Seven lambs were born to seven ewes. One lamb was abandoned after birth and the other six lambs developed

bronchopneumonia. Lambs displaying severe clinical signs of respiratory disease were humanely euthanized. All lambs that died naturally were recovered within 18 h. We necropsied carcasses and tested tissues via the culture and PCR methods described earlier.

Gross lesions of bronchopneumonia included consolidation of the cranioventral lung lobes, scattered necrotic foci within affected lung tissue, and fibrinous pleuritis, with up to 75% of each lung affected. Histologic lesions included suppurative bronchopneumonia with varying evidence of leukocytolysis, typical of pasteurellosis and caused by *lkt+* *Pasteurellaceae* bacteria (Gilmour and Gilmour 1989; Jeyaseelan et al. 2002; Dassanayake et al. 2009). Additionally, we noted varying evidence of lymphocytic cuffing of bronchioles, bronchial epithelial cell hyperplasia, and alveolar histiocytosis typically associated with mycoplasmosis (Besser et al. 2008; Nicholas et al. 2008). Multifocal hepatocellular necrosis and suppuration were seen in three cases and suggested systemic infection. Three lambs had evidence of fibrinous peritonitis and/or pericarditis, lesions often associated with hemorrhagic septicemia caused by *Pasteurella multocida* (Carter and De Alwis 1989). Splenic and thymic lymphoid depletion were present in most cases and suggested chronic stress or disease. Lambs with pneumonia also consistently had suppurative otitis media, a condition associated with *Mycoplasma bovis* in dairy calves (Maunsell et al. 2012) and *Pasteurellaceae* in domestic lambs (Macleod et al. 1972; Jensen et al. 1982).

Lambs that died during 2013 showed similar clinical, necropsy, and diagnostic patterns to lambs from 2014 (Table 1). These observations also resembled those in dead lambs recovered from free-ranging bighorn sheep herds in Colorado. Overall, younger captive-born lambs (age 11–15 d;  $n=5$ ) consistently had evidence of bronchopneumonia with leukocytolysis and mild peribronchiolar infiltrates of lymphocytes and plasma cells, suggestive of pasteurellosis, and lacked peribronchiolar lymphocytic cuffing suggestive of mycoplasmosis. These younger lambs had a short clinical disease course character-

TABLE 1. Results of postmortem examination of bighorn (*Ovis canadensis*) lambs including bacterial culture, PCR, and a brief summary of pathology. All lambs had suppurative bronchopneumonia and were born in 2013 or 2014 to ewes (denoted by first two digits of lamb identification) brought into captivity in 2013 from a poorly performing herd in Gribbles Park, Colorado, USA, for a study on neonatal respiratory diseases.<sup>a</sup>

Lamb identification	Days alive	Lung	Liver	Middle ear (bulla)	Nasal swab	Tonsil swab	Pathology
51-G3 <sup>b</sup>	11	Bt, Mo	ND	—	Mo	Bt	Leukocytolysis and minimal peribronchiolar lymphoplasmacytic infiltrates, fibrinous pleuritis, otitis media
80-G3 <sup>b</sup>	12	Bt, Mo	—	—	—	—	Leukocytolysis, necrosuppurative hepatitis
87-G3	14	Mo	Pm	ND	Ms, Mo	Ms	Leukocytolysis and mild peribronchiolar infiltrates of lymphocytes and plasma cells, otitis media
80-G4	15	Bt	Bt	Bt	Mo	Bt	Leukocytolysis and mild peribronchiolar infiltrates of lymphocytes and plasma cells, otitis media
87-G4	15	Bt	Bt	ND	Mo	Bt	Leukocytolysis, otitis media
95-G3	23	Mo, Pm	Mo, Pm	—	Mo	ND	Leukocytolysis and peribronchiolar lymphocytic cuffs, necrosuppurative hepatitis, fibrinous pleuritis, fibrinous peritonitis
11-G4	29	Bt, Mo	Bt	Bt	Mo	Bt	Leukocytolysis, fibrinous pleuritis
82-G4	34	Mo	—	Mo, Pm	Mo	Ms	Leukocytolysis, peribronchiolar lymphocytic cuffs, and bronchiolar epithelial hyperplasia, otitis media
51-G4	36	Mhg, Pm	Mhg, Pm	ND	Mo	Mhg, Pm	Leukocytolysis and peribronchiolar lymphocytic cuffs, fibrinous pleuritis, fibrinous pericarditis, otitis media
91-G3 <sup>b</sup>	43	Bt, Ms	Bt	Bt	Mo	Bt	Leukocytolysis and peribronchiolar lymphocytic cuffs, alveolar histiocytosis, fibrinous pleuritis, necrosuppurative hepatitis, otitis media
82-G3	57	Pm, Mo	Pm	Pm	Mo	ND	Peribronchiolar lymphocytic cuffs and bronchiolar epithelial hyperplasia, otitis media
55-G4	68	Bt, Mo, Pm	Bt, Pm	Mo	Mo	Bt	Peribronchiolar lymphocytic cuffs and bronchial epithelial hyperplasia, fibrinosuppurative pericarditis

<sup>a</sup> G3 = born in 2013; G4 = born in 2014; Bt = Lkt+ *Bibersteinia trehalosi*; Mo = *Mycoplasma ovipneumoniae*; Pm = *Pasteurella multocida*; Mhg = Lkt+ *Mannheimia haemolytica* or *M. glucosida*; Ms = Lkt+ *Mannheimia* sp.; ND = no pathogen detected; — = not sampled.

<sup>b</sup> Dam was treated with tildipirosin prior to lambing.

ized by abrupt onset of lethargy followed rapidly by death. In contrast, older lambs (age 57–68 d;  $n=2$ ) had a prolonged disease course characterized by coughing, nasal discharge,

and ear drooping/head shaking. These older lambs lacked lesions of leukocytolysis in the lungs, but had prominent peribronchiolar lymphocytic cuffs and bronchial epithelial

hyperplasia suggestive of mycoplasmosis. In these lambs, suppurative bronchopneumonia was observed despite the lack of leukocytolysis and may have been due to infection with nonleukotoxigenic *Pasteurellaceae* (such as *P. multocida*). Lambs age 23–43 d ( $n=5$ ) had mixed pathology with clinical signs of coughing, nasal discharge, and head shaking.

Bacterial culture and PCR assays for *M. ovipneumoniae* and *Pasteurellaceae* demonstrated both pathogen groups in all lamb pneumonia cases examined (Table 1). Combined pathology and diagnostic results suggested both groups of agents contributed to poor lamb recruitment. Otitis media did not appear to be associated strictly with one pathogen based on bacteriology results (Table 1). For each of three cases with fibrinous pericarditis and/or peritonitis *P. multocida* was detected in the lung and liver, reminiscent of hemorrhagic septicemia in other species.

Bacterial agents consistently identified in the ewes included lkt+ *Pasteurellaceae* (predominantly *B. trehalosi*), nonleukotoxigenic *Pasteurellaceae* (predominantly *P. multocida*), and *M. ovipneumoniae* (Supplementary Material Table S1). Adult ewes that died during the course of this study all had chronic sinusitis and chronic bronchopneumonia with evidence of sinus tumors (Fox et al. 2011). These tumors may facilitate bacterial pathogen persistence in sinuses, leading to a chronic carrier state (Fox et al. 2015). This combination of pathogens also has been observed in other free-ranging bighorn sheep herds in Colorado showing similar patterns of poor recruitment (Miller and Wolfe 2011; Miller et al. 2013).

Although the combination of agents appeared homogenous across the captive ewes, lambs died from a spectrum of disease ranging from acute pasteurellosis-type disease and lesions in young lambs to chronic mycoplasmosis-type disease and lesions in older lambs. These findings suggest that when lamb carcasses are evaluated from free-ranging herds, the timing of sampling may bias investigators to conclude that one pathogen or another predominates as a source of

mortality when, perhaps, the combination of pathogens is more important at a herd level. Alternatively, the roles of lkt+ *B. trehalosi* and *P. multocida* in lamb pneumonia could be relatively underrecognized or geographically limited to Colorado bighorn sheep herds.

We suspect that herds with a similar history to the Gribbles Park bighorn sheep have little chance for improvement through treatment or selective culling. Our results suggest that some bighorn sheep populations with respiratory disease can reach a state of chronic persistent infection that cannot be cleared. In these instances, removing the herd unit may be the best management option. We recommend developing earlier and more aggressive management intervention strategies to prevent herds from reaching this chronically infected state.

Our work has been supported by the Wyoming Game and Fish Department, Colorado Parks and Wildlife, the Wyoming Governors Big Game License Coalition, and the Wyoming Wild Sheep Foundation. Research was approved through the Wyoming Game and Fish Animal Care and Use Committee. We thank M. Huizenga, C. Hansen, and S. Lockwood for their hard work in care of captive sheep and data collection. We also appreciate field and logistic assistance from Colorado Parks and Wildlife staff in acquiring captive bighorn sheep, in particular J. Aragon, J. Grigg, K. Woodruff, B. Dreher, D. Prenzlou, and L. Wolfe, as well as the Colorado Parks and Wildlife Commission's approval of transferring these bighorn sheep to captivity. Additionally, we thank S. Srikumaran and B. Raghavan for their role in early research objectives and acquisition of sheep.

#### SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/2016-05-097>.

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Submitted for publication 6 May 2016.

Accepted 30 June 2016.