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A Bighorn Sheep Die-off in Southern Colorado Involving a *Pasteurellaceae* Strain that May Have Originated from Syntopic Cattle

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ABSTRACT: We investigated a pasteurellosis epizootic in free-ranging bighorn sheep (*Ovis canadensis*) wherein a *Pasteurellaceae* strain carried by syntopic cattle (*Bos taurus*) under severe winter conditions appeared to contribute to pneumonia in affected bighorns. Twenty-one moribund or dead bighorn sheep were found on the "Fossil Ridge" herd's winter range, Colorado, USA, between 13 December 2007 and 29 February 2008. Eight carcasses examined showed gross or microscopic evidence of acute to subacute fibrinous bronchopneumonia. All eight carcasses yielded at least one β-hemolytic *Mannheimia haemolytica* biogroup $1^{(\pm G)}$ strain, and seven also yielded a β-hemolytic *Bibersteinia trehalosi* biogroup 4^{CDS} strain; evidence of *Pasteurella multocida*, *Mycoplasma ovipneumoniae*, and parainfluenza 3 and bovine respiratory syncytial viruses was also detected. Isolates of β-hemolytic *Mannheimia haemolytica* biogroup 1^{G} from a bighorn carcass and a syntopic cow showed 99.5% similarity in genetic fingerprints; *B. trehalosi* biogroup 4^{CDS} isolates were ≥94.9% similar to an isolate from a nearby bighorn herd. Field and laboratory observations suggested that pneumonia in affected bighorns may have been caused by a combination of pathogens including two pathogenic *Pasteurellaceae* strains—one likely of cattle origin and one likely of bighorn origin—with infections in some cases perhaps exacerbated by other respiratory pathogens and severe weather conditions. Our and others' findings suggest that intimate interactions between wild sheep and cattle should be discouraged as part of a comprehensive approach to health management and conservation of North American wild sheep species.

Key words: *Bibersteinia trehalosi*, bighorn sheep, cattle, pneumonia, *Mannheimia haemolytica*, *Mycoplasma*, *Ovis canadensis*, *Pasteurella multocida*.

The decline of bighorn sheep (*Ovis canadensis*) abundance throughout much of western North America appears attributable to historical overharvest, habitat loss or degradation and, in large part, to epizootics caused by introduced pathogens, some of which have now become enzootic. The earliest reports of epizootics in bighorn sheep (e.g., accounts in Warren, 1910; Grinnell, 1928; Shillinger, 1937; Honess and Frost, 1942) closely followed the advent of domestic livestock grazing in bighorn habitat, suggesting that bighorn populations in some areas first may have been exposed to novel pathogens in the 1800s. More than a century later, recurring respiratory disease epizootics remain obstacles to recovering bighorn sheep populations to historic levels (Miller, 2001). Understanding and, where feasible, controlling specific risk factors that may cause or precipitate pneumonia epizootics in bighorn sheep has become an imperative of this species' conservation. Unfortunately, post hoc investigations of epizootics under field conditions rarely yield clear answers regarding source(s) of the responsible pathogen(s) and the role of potential contributing stressors like weather. Here, we describe a case wherein exposure to a pathogen carried by syntopic cattle (*Bos taurus*) under severe winter conditions may have contributed to the onset of epizootic pasteurellosis in a free-ranging bighorn herd. Our objectives are to report the findings of our field and laboratory investigations of this epizootic and to broaden conventional thinking about risk factors that may affect the health and perpetuation of North American wild sheep species.

The "Fossil Ridge" bighorn herd in

southern Colorado, USA (38°30–41′N, 106°34–48′W) was started with a translocation of 20 individuals and had grown to >60 animals by 2006 (George et al., 2009). Available range was restricted during most winters and recreation activity may have further reduced the area occupied by bighorns. As a likely consequence of limited winter range, a local Hereford breed cattle rancher reported that for about 15 yr some bighorns had come into his cattle feed lines on private land at times during fall and winter. On the basis of the belief that such interactions were not particularly risky to bighorn sheep, this behavior was not discouraged by local wildlife managers.

The winter of 2007–08 was one of the most severe in recorded history for the Gunnison Basin (Colorado Division of Wildlife, 2009), an area that included the Fossil Ridge herd's range. On the basis of data compiled at the Gunnison County Electric Association weather station for the United States National Oceanic and Atmospheric Administration, about 51 cm of heavy, wet snow fell during 6–7 December 2007, burying mountain shrub communities across the basin; below-average temperatures ranging from −7 C to −20 C precluded any appreciable snowmelt thereafter. Apparently healthy bighorn sheep were seen on traditional winter range during the week of 16 December, although no lambs from the previous summer were observed.

The epizootic at Fossil Ridge was first reported on 23 December 2007 by the local rancher, who noticed fewer bighorns in the area and subsequently found three carcasses and two sick animals nearby. Clinical signs included depression, thick nasal discharge, and dyspnea, but little coughing. One sick animal was shot; the other was found dead the next day. Subsequent field investigation on 23 and 24 December revealed additional carcasses and sick animals. In the course of discussing the situation, the rancher mentioned finding an adult female bighorn carcass 10 days earlier. He also noted that this had been a particularly bad year for respiratory disease problems in his cattle herd, perhaps because recently purchased replacement animals had "brought something in" (e.g., Frank et al., 2003); however, no previous diagnostic work had been done on the cattle herd.

Twenty-one moribund or dead bighorn sheep were found on the Fossil Ridge herd's winter range between 13 December 2007 and 29 February 2008; three additional carcass remains were found in October 2008. Eight relatively intact carcasses were necropsied; other carcasses were too scavenged, decomposed, or inaccessible to examine. Lung, tonsil, and other select tissues were submitted to the Caine Veterinary Teaching Center (CVTC; University of Idaho, Caldwell, Idaho, USA) for bacterial culture with emphasis on *Pasteurellaceae* (modified from Jaworski et al., 1998), to Microbial Research, Inc. (MRI; Fort Collins, Colorado, USA) and the Washington Animal Disease Diagnostic Laboratory (Pullman, Washington, USA) for *Mycoplasma* spp. culture, and to the CVTC, MRI, and the Colorado State University Veterinary Diagnostic Laboratory (CSUVDL; Fort Collins, Colorado, USA) for PCR assays to detect *Mycoplasma* spp. DNA (Baird et al., 1999; Besser et al., 2008; D. Bade, unpubl. data; G. Weiser, pers. comm.). Antibody titers to parainfluenza 3 (PI3; recent or active infection titer ≥1:256) virus and bovine respiratory syncytial virus (BRSV; recent or active infection titer ≥1:64) were measured by virus neutralization tests at the CSUVDL. Select representative *Pasteurellaceae* isolates from carcasses and live animals (sampling detailed below) were further compared by repetitive DNA sequence genotyping by Newport Laboratories (Worthington, Minnesota, USA) using PCR and boxA1R primer (Goldberg et al., 2006). Select *Mycoplasma* spp. isolates were identified by DNA sequencing at the University of Minnesota Veterinary Diagnostic Labora-

TABLE 1. Respiratory disease agents detected from dead and surviving bighorn sheep (*Ovis canadensis*) and syntopic cattle (*Bos taurus*) during and after the December 2007 epizootic at "Fossil Ridge" in southwestern Colorado, USA. Evidence of infection or exposure came from culture data for *Pasteurellaceae*, from culture and PCR data for mycoplasmas, and from serology data for the two respiratory viruses. See text for methods and interpretation.

Agent	Bighorn sheep			Cattle
	Dead (December 2007), $n=8$	Alive (February–March 2008), $n=10$	Alive (February 2009), $n=11$	Alive (February 2008), $n=27$
Mannheimia haemolytica				
Biogroup 1 (β)[a]	5[b]	0	0	0
Biogroup 1^{G} (β)	7	0	0	1
Biogroup 1^{AG} (β)	1	0	0	0
Biogroup 3 (β)	0	0	2	0
Biogroup 3^{A}	0	0	1	0
Biogroup $16^{AG(\pm E)}$	0	0	0	20
Bibersteinia trehalosi				
Biogroup 4^{CDS} (β)	7	1	0	0
Biogroup $4^{(\pm various)}$	0	5	2	0
Biogroup $2^{(\pm various)}$	0	3	1	0
Pasteurella multocida	5	4	1	0
Mycoplasma spp.	8	3	1	15
Mycoplasma ovipneumoniae	8	sd[c]	sd	9
Mycoplasma bovirhinis	0	sd	sd	9
Bovine respiratory syncytial virus (titer ≥1:64)	2 (of 2)	2	nr[d]	8
Parainfluenza 3 virus (titer ≥1:256)	2 (of 2)	2	nr	19

[a] Isolates showed β-hemolysis on blood agar.

[b] Number of individuals positive; total sample size is shown in the column heading except where noted.

[c] Samples discarded by reference laboratory before species-specific PCR being performed.

[d] Not reported because prior vaccination confounded interpretation of titers.

tory (UMVDL; Saint Paul, Minnesota, United States). Select liver trace mineral concentrations were measured at the CSUVDL using established methods (Rosen et al., 2009) to rule out feed-associated intoxication.

All carcasses examined showed gross and microscopic evidence of acute to subacute fibrinous bronchopneumonia. The predominant microscopic lesion was severe, subacute bacterial bronchopneumonia associated with oat-shaped macrophages, edema, fibrin, and a few neutrophils filling and expanding alveolar spaces. Three dominant β-hemolytic *Pasteurellaceae* strains were recovered (Table 1). All eight carcasses yielded at least one *Mannheimia haemolytica* strain, including biogroup 1^{G} isolates from seven and biogroup 1 isolates (<83% genetic fingerprint similarity to the biogroup 1^{G} strain) from five. A *Bibersteinia trehalosi* biogroup 4^{CDS} strain also was isolated from seven carcasses. In addition, *Pasteurella multocida* (subsp. b or biotype U) was isolated from five carcasses. Lung or tonsil tissue samples from all eight bighorn carcasses tested PCR positive for *Mycoplasma ovipneumoniae* (Table 1). Serology in two cases where blood was available also suggested exposure to PI3 and BRSV. Liver tissue mineral concentrations (mean±95% confidence interval; range) for copper (146.1±78.6 parts per million [ppm] dry weight; 12.1–316 ppm), manganese (6.7±1.4 ppm; 3.8–9.7 ppm), molybdenum (3.7±1.2 ppm; 1.9–5.8 ppm), and zinc (122.7±37.3 ppm; 60.3–208 ppm)

were all within acceptable limits (Rosen et al., 2009; CSUVDL, unpubl. data; L. L. Wolfe, unpubl. data), but selenium concentrations (0.7±0.2 ppm; 0.5–1.5 ppm) were lower than reported for healthy bighorns (Rosen et al., 2009).

We captured 10 of the 11 known surviving bighorns (nine adult females and one adult male) via darting about 5 or 9 wk after the die-off was first reported. Two adult females were equipped with very-high-frequency radiocollars and the other eight animals were marked with unique plastic ear tags. We collected blood and oropharyngeal swabs and treated each animal with tulathromycin (DRAXXIN®, Pfizer Animal Health, New York, New York, USA), doramectin (DECTOMAX®, Pfizer Animal Health), and a commercial vaccine containing killed PI3, BRSV, infectious bovine rhinotracheitis virus, and bovine viral diarrhea virus (Triangle 4, Fort Dodge; Fort Dodge, Iowa, USA). In addition, we collected blood and triplicate nasopharyngeal swabs from a subset of the rancher's cattle ($n=27$) and treated all of the syntopic cattle with tulathromycin ($n=70$). We placed swabs in Port-A-Cul™ tubes (Becton, Dickinson and Company, Sparks, Maryland, USA) and hand-delivered one set to MRI and shipped the other overnight to the CVTC for culture and *Mycoplasma* spp. PCR. A third swab in brain–heart infusion broth was submitted to CSUVDL for *Mycoplasma* spp. PCR. Serum antibody titers to PI3 and BRSV were measured by serum neutralization (CSUVDL).

Nonhemolytic strains of *B. trehalosi* biogroups 2 and 4^{CDS} and *P. multocida* were the primary *Pasteurellaceae* isolated from the surviving bighorns (Table 1); *Mannheimia haemolytica* was not isolated. Nonhemolytic *M. haemolytica* biogroup $16^{AG(\pm E)}$ were the most abundant *Pasteurellaceae* isolated from cattle, although a β-hemolytic *M. haemolytica* biogroup 1^{G} also was isolated from one of the cattle (Table 1); *B. trehalosi* were not isolated from cattle. Three of the surviving bighorns and 15 of the sampled cattle were PCR positive for *Mycoplasma* spp. (Table 1); both *Mycoplama ovipneumoniae* and *Mycoplasma bovirhinis* were detected in the cattle by PCR or culture. A proportion of both the surviving bighorns and the sampled cattle had antibody titers suggesting exposure to PI3 and BRSV (Table 1).

In February 2009, surviving Fossil Ridge bighorn sheep were again baited and recaptured via drop netting. Of 11 animals captured, three (one lamb, two adult females) were unmarked and thus had not been handled in 2008. We sampled and tested bighorns as above and treated each with tulathromycin, doramectin, and two commercial vaccines, Triangle 4 and a *Mannheimia haemolytica* type A1 bacterin-toxoid (One Shot®, Pfizer Animal Health). Cultures yielded β-hemolytic *M. haemolytica* biogroup 3 (Table 1), along with nonhemolytic *B. trehalosi*, *M. haemolytica*, and *P. multocida* isolates. On the basis of numbers of non-*Pasteurellaceae* recovered, shipping and processing delays likely biased culture results. Only the lamb was PCR positive for *Mycoplasma* spp. Cattle were not resampled.

Laboratory findings linked a combination of pathogens to this epizootic. Despite sampling lags and some heterogeneity among the *Pasteurellaceae* isolated from pneumonic bighorns, a β-hemolytic, *M. haemolytica* biogroup 1^{G} isolate from a bighorn carcass showed 99.5% similarity in its genetic fingerprint to the *M. haemolytica* biogroup 1^{G} isolate from one of the syntopic cattle; moreover, these two isolates' fingerprints were ≥95.5% similar to fingerprints of other *M. haemolytica* biogroup 1^{G} isolates from temporally and geographically separate cases of domestic sheep (*Ovis aires*)-associated acute pasteurellosis in bighorns (Foreyt, 1989; George et al., 2008). These findings support the notion that domestic ruminants can harbor *Pasteurellaceae* strains

that are pathogenic in bighorn sheep. The β-hemolytic *B. trehalosi* biogroup 4^{CDS} also isolated from most pneumonic Fossil Ridge bighorns (but none of the syntopic cattle) has been recovered from several Colorado bighorn herds (Green et al., 1999; L. L. Wolfe and M. W. Miller, unpubl. data); *B. trehalosi* biogroup 4^{CDS} isolates from both dead and surviving Fossil Ridge bighorns were ≥94.9% similar by genetic fingerprinting to isolates from the nearby Taylor River bighorn herd where this strain (called "ribotype E_{CO}" elsewhere; Green et al., 1999) has been enzootic since at least the early 1990s (M. W. Miller and L. L. Wolfe, unpubl. data). These findings support the notion that enzootic *Pasteurellaceae* also can contribute to pneumonia during epizootics in bighorn sheep. In addition to *Pasteurellaceae*, both bighorns and syntopic cattle showed evidence of exposure to *Mycoplasma ovipneumoniae* (most likely of bighorn origin), PI3, and BRSV.

On the basis of findings from necropsy and live animal sampling, we believe that this pneumonia epizootic was caused by a combination of pathogens including two or more pathogenic strains of *Pasteurellaceae*—a *Mannheimia haemolytica* strain most likely of cattle origin and a *B. trehalosi* strain most likely of bighorn origin—with some cases perhaps exacerbated by exposure to *Mycoplasma* spp. and viruses of cattle or bighorn origin. Despite what we believe to be compelling support for this explanation, however, we recognize that identifying the true cause(s) of this and other pasteurellosis epizootics in bighorn sheep retrospectively under field conditions cannot be done with certainty. For example, interpretation of culture data is complicated by the heterogeneity and dynamics of *Pasteurellaceae* in bighorns and in domestic sheep and cattle (Miller et al., 1997; Jaworski et al., 1998; Miller, 2001; Safaee et al., 2006; Kelley et al., 2007; George et al., 2008; Tomassini et al., 2009), and is further confounded by influences of sample handling and laboratory methods (Safaee et al., 2006; George et al., 2008; Dassanayake et al., 2009a; L. L. Wolfe, unpubl. data) and the potential for pathogenicity to change within strains via horizontal transfer of the gene encoding leukotoxin (Kelley et al., 2007). In addition to the pathogens we detected, weather conditions may have contributed at Fossil Ridge either as a stressor on bighorns or cattle, or simply by increasing interactions between bighorns and cattle (Fig. 1). Notably, however, we did not observe epizootic pasteurellosis in a bighorn herd wintering in the nearby Taylor River drainage despite equally severe winter conditions and the presence of several pathogens also present in the Fossil Ridge herd (β-hemolytic *B. trehalosi* biogroup 4^{CDS}, PI3, BRSV, *Mycoplasma ovipneumoniae*; L. L. Wolfe, unpubl. data), suggesting that the presence of *Mannheimia haemolytica* or *Mycoplasma bovirhinis* in syntopic cattle may have helped trigger the Fossil Ridge epizootic.

FIGURE 1. The intensity and duration of interactions between bighorn sheep and cattle on feed lines during December 2007 may have contributed to the apparent exchange of respiratory pathogens associated with a pasteurellosis epizootic in the "Fossil Ridge" bighorn herd that resided in southern Colorado, USA.

Segregating wild sheep from domestic sheep has long been recognized as important to preventing epizootics in bighorn sheep (Warren, 1910; Shillinger, 1937; Foreyt and Jessup, 1982). Thus far, similar emphasis has not been placed on prevent-

ing interactions between cattle and bighorn sheep, most likely because species differences and a tendency toward interspecies avoidance are thought to help minimize opportunities for pathogen exchange (Foreyt and Lagerquist, 1996). However, the similarities between *Pasteurellaceae* and other respiratory pathogens of cattle and domestic sheep suggest similar adverse consequences to bighorn sheep if pathogen transmission were to occur between cattle and bighorns (Onderka et al., 1988; Singer et al., 2000). Such consequences have been demonstrated experimentally: five of eight bighorns died within 4 days of receiving intradermal injections of a cattle vaccine containing attenuated, live *Mannheimia haemolytica* (Onderka et al., 1988), four bighorns died within 2 days after intratracheal inoculation with *M. haemolytica* isolated from cattle (Dassanayake et al., 2009b), and one of five captive bighorns died 6 days after being copastured with Holstein calves (Foreyt and Lagerquist, 1996). We conclude from our findings, combined with other published observations, that intimate interactions between wild sheep and cattle (e.g., shared feed lines or troughs) also should be discouraged as part of a comprehensive approach to health management and conservation of North American wild sheep species.

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