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Assessing Timing and Causes of Neonatal Lamb Losses in a Bighorn Sheep (*Ovis canadensis canadensis*) Herd via Use of Vaginal Implant Transmitters

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ABSTRACT: We evaluated the use of vaginal implant transmitters (VITs) as a means of detecting, capturing, and radio collaring Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) lambs to estimate survival and to facilitate carcass recovery to assess causes of mortality. We focused on one of several bighorn herds in Colorado, US, suffering from depressed recruitment that was not preceded by a classic all-age die-off. We captured, radio-collared, diagnosed pregnancy by ultrasound examination, and inserted VITs into 15 pregnant ewes from a herd residing near Granite, Colorado. We were subsequently able to collar a lamb from each of 13 VITs, and two additional lambs opportunistically from ewes without transmitters. As lambs died, we recovered and submitted carcasses for necropsy and laboratory assessment. All lambs captured and one additional lamb (carcass found opportunistically) were dead by about 130 d of age: 11 died of apparent pneumonia (all within 8–10 wk of age), one died from trauma after being kicked or trampled, one was killed by a mountain lion (*Puma concolor*), and three died of starvation likely caused by abandonment after capture. Pneumonic lambs had involvement of *Mycoplasma ovipneumoniae* and leukotoxigenic *Bibersteinia trehalosi*. The use of VITs and lamb collars enabled us to efficiently identify pneumonia as the predominant cause of depressed lamb recruitment in this herd; however, we urge care in neonatal lamb handling to minimize abandonment.

Key words: Bibersteinia trehalosi, bighorn sheep, *Mycoplasma ovipneumoniae*, *Ovis canadensis*, *Pasteurellaceae*, recruitment, respiratory disease, vaginal implant transmitters.

Respiratory disease–associated “all-age” die-offs affect Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) herds with variable frequency and severity in Colorado, US, and elsewhere in western North America (George et al. 2008; Wolfe et al. 2010; Butler et al. 2013). Years of depressed recruitment often follow these die-offs, adding to population suppression and hindering recovery (e.g., George et al. 2008; Wolfe et al. 2010; Butler et al. 2013). In recent years, bighorn herds in Colorado and elsewhere also have suffered extended periods of depressed lamb recruitment in the apparent absence of a classic all-age die-off (e.g., Sirochman et al. 2012; Smith et al. 2014a). In these herds lamb pneumonia is suspected but often unconfirmed.

Respiratory disease is a common cause of lamb mortality among Rocky Mountain bighorn herds, but predators, poor nutrition, weather, and trauma also kill bighorn lambs (Hass 1989; Butler et al. 2013; Smith et al. 2014a). Assessing causes of poor lamb recruitment is difficult when few carcasses are available for postmortem evaluation. Investigating such problems via observation-based or opportunistic sampling may yield biased or misleading data (Smith et al. 2014a).

Here we describe a field study evaluating the feasibility of using vaginal implant transmitters (VITs; Bishop et al. 2011; Smith et al. 2014b) as a means of detecting, capturing, and radio-collaring bighorn lambs to estimate survival and facilitate carcass recovery to assess causes of mortality. Our work focused on one of Colorado’s bighorn herds with depressed recruitment that apparently was not preceded by a classic all-age die-off.

The “Granite” bighorn sheep herd occupies winter range about 24 km north of Buena Vista, Colorado (39°2′48″N, 106°15′44″W; elevation 2,780 m). This herd began with translocation of 17 adult bighorns in 1978 and

TABLE 1. Numbers of lambs and ewes observed at the Granite Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) herd winter range from 2005 to 2016, Granite, Colorado, USA. Count data were derived from several surveys conducted each winter, from a helicopter and on the ground, during January–March. Reported numbers are the highest single-day counts of rams (included in "herd size"), ewes, and lambs recorded during each winter. Lamb/100 ewe ratios were calculated from the high single-day lamb and ewe counts to provide an index of relative annual recruitment (birth to winter), which declined abruptly beginning in 2011–12.

Winter	Herd size (minimum)	Lambs	Ewes	Lambs/100 ewes (calculated)[a]
2005–06	63	15	34	44
2006–07	66	12	38	32
2007–08	46	13	42	31
2008–09	67	15	48	31
2009–10	78	18	42	43
2010–11[b]	101	20	58	34
2011–12	67	0	54	0
2012–13	46	8	34	24
2013–14	41	1	35	2
2014–15	39	3	30	10
2015–16	44	2	28	7
2016–17	32	7	20	35

[a] Calculated as (highest number of lambs observed/highest number of ewes observed)×100.

[b] Clinical respiratory disease first noted in winter, during February 2011. See text for details.

reached an estimated peak size of 101 animals in 2011 (Table 1). Annual lamb recruitment from birth (May–June) until winter (December–February; hereafter, "recruitment to winter") generally remained >30 lambs/100 ewes prior to 2011 based on winter surveys (Table 1).

In February 2011, clinical respiratory disease characterized by paroxysmal coughing, mucopurulent nasal discharge, and head shaking was noted among both sexes and all age classes in a small portion of the bighorns observed on this winter range. We detected *Mycoplasma ovipneumoniae* and leukotoxigenic *Pasteurellaceae*, particularly *Bibersteinia trehalosi*, in the herd by culture and PCR of nasal/oropharyngeal swabs (Wood et al. 2017). Serology demonstrated exposure to parainfluenza 3 virus but not to respiratory syncytial virus. Clinical signs largely abated by spring, and no bighorn carcasses were encountered during the time when clinical respiratory disease was observed. However, a few adult and yearling losses may have occurred in the months afterward based on the reduced number of adult bighorns seen at Granite during winter 2012 (Table 1). No lambs born in 2012 survived to the following winter and recruitment to winter was poor though 2014–15 (0–8 lambs annually; Table 1).

In February 2013 we darted (air-powered rifle, DanInject™, Børkop, Denmark; 3 cc type U dart, Pneudart, Williamsport, Pennsylvania, USA) adult female bighorns from the ground with a combination of 33 mg butorphanol, 10 mg azaperone, and 12 mg medetomidine (BAM, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA). Pregnancy status was determined with transabdominal ultrasonagraphy (Sonovet 2000, Universal Medical Systems, Bedford Hills, New York, USA). Fifteen pregnant ewes (estimated 3.5–>5.5 yr old) were fitted with radio collars (Advanced Telemetry Systems, Insanti, Minnesota, USA) and VITs (Advanced Telemetry Systems) using techniques described by Bishop et al. (2011). At the time of capture ewes were treated with 15 mg doramectin (Dectomax, Zoetis, Pasippany, New Jersey, USA), 3 mg selenium-tocopherol (Bo-Se, Schering Plough, Kenilworth, New Jersey, USA) and 1,200 mg oxytetracycline (Liquamycin® LA200®, Zoetis) by subcutaneous injections in an attempt to reduce respiratory pathogen burden and provide supportive care. Essentially the same respiratory pathogen profile was detected, by similar methods, as in 2011. Although our sample size was limited, it represented >40% of the females known to be on the Granite winter range in 2013 (Table 1) and thus seemed sufficient for determining the predominant causes of lamb mortality.

We monitored radio collars and VITs weekly until mid-April, then daily until VITs were expelled. We tracked the expelled VITs and radio-collared ewes from the ground to locate the birth sites and lambs. We captured newborn lambs by hand, fitted each with an expandable, breakaway radio collar (M4210;

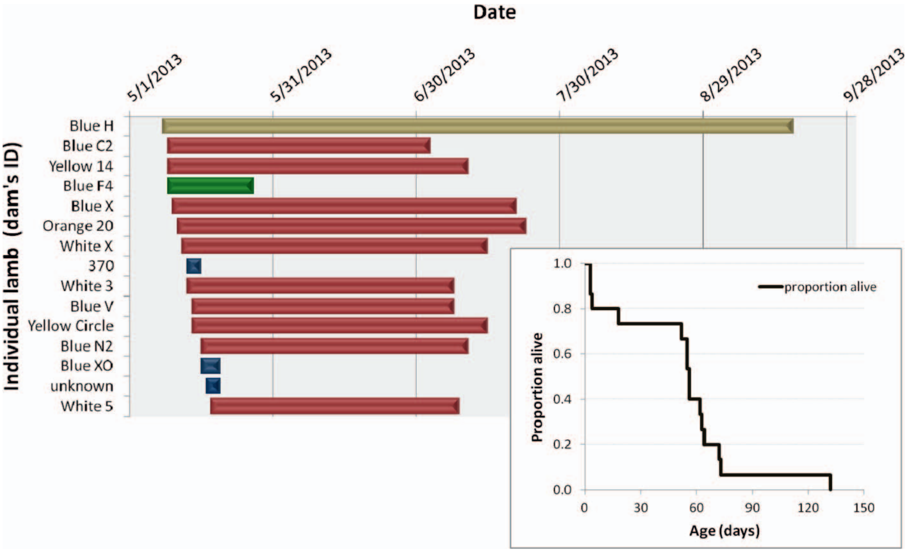

FIGURE 1. Timing and cause of death for radio-collared Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) lambs (n=15) captured as neonates during May 2013, Granite, Colorado, USA. Most lambs died from suppurative bronchopneumonia (red), but three were abandoned (blue), one was trampled (pale green), and one was killed by a mountain lion (*Puma concolor*; tan). Inset: Proportion of collared lambs surviving by age. All pneumonia cases occurred during the period of sharpest decline between 52 d of age and 73 d of age.

Advanced Telemetry Systems), and released them. One VIT was expelled in early April and was not at a birth site. The other 14 were expelled at birth sites and we found a lamb at each site within 48 hr. We captured and collared 13 of those 14 lambs, along with two additional newborn lambs from ewes without VITs that we encountered while tracking a VIT. Outcomes exceeded our expectations with respect to feasibility and were comparable to experiences using similar approaches elsewhere (Smith et al. 2014b).

Birth dates ranged from 8 May–19 June (median 14 May; Fig. 1). Dead lambs with collars (n=15) were recovered within 12–48 hr after receiving a mortality signal. One additional uncollared lamb carcass also was recovered. Carcasses were necropsied within 48 hr of mortality signal detection. All 15 collared lambs were dead by 130 d of age (Fig. 1): 10 died of apparent pneumonia within 8–10 wk of age (Table 2 and Fig. 1), one died from trauma after being kicked or trampled, one was killed by a mountain lion (*Puma concolor*), and three died of starvation likely caused by abandonment after capture. The additional intact lamb carcass found opportunistically also had gross evidence of pneumonia. The timing of pneumonia cases strongly coincided (Fig. 1).

Lambs with pneumonia consistently had cranioventral consolidation and scattered necrotic foci within the lungs, with up to 75% of each lung affected. Nine of the 11 lambs with gross evidence of pneumonia were examined histologically. Each had suppurative to necrosuppurative bronchopneumonia with variable lesions including lymphocytic cuffing of bronchioles, bronchiolar epithelial hyperplasia, and alveolar histiocytosis (lesions typically associated with *M. ovipneumoniae*); and prominent leukocytolysis (typically associated with leukotoxigenic *Pasteurellaceae*). Although lesions associated with *Pasteurellaceae* predominated in the youngest lambs (52–56 d), most cases had lesions suggesting mixed bacterial infections. Each of the eight pneumonic lambs assayed via bacterial culture and PCR for *M. ovipneumoniae* and *Pasteurellaceae* yielded both pathogens (Table 2). The *Pasteurellaceae* bacteria most often identified were leukotoxigenic *B. trehalosi*. Lungworm

TABLE 2. Key postmortem findings from pneumonic Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) lamb carcasses (*n*=11) collected during July–August 2013, Granite, Colorado, USA. Reported results include bacterial culture, PCR, and brief pathology summary. All lambs had suppurative bronchopneumonia. Compare to Wood et al. (2017).

Dam ID	Days alive	Lung[a]	Liver[a]	Bulla[a]	Pathology
White 5	52	Bt Mo	Bt	X	Leukocytolysis and minimal peribronchiolar lymphoplasmacytic infiltrates, fibrinous pleuritis, otitis media
Blue C2	55	X	X	X	Leukocytolysis and minimal peribronchiolar lymphoplasmacytic infiltrates, necrotizing hepatitis, otitis media
Unknown	~55[b]	X	X	X	Leukocytolysis, fibrinous pleuritis; moderate autolysis may have obscured subtle histologic lesions
Blue V	55	Bt Mo	Bt	X	Leukocytolysis and minimal peribronchiolar lymphoplasmacytic infiltrates, fibrinous pleuritis, otitis media
White 3	56	Bt Mo	Bt	X	Leukocytolysis and minimal peribronchiolar lymphoplasmacytic infiltrates, fibrinous pleuritis, necrotizing hepatitis, otitis media
Blue N2	56	Bt Pm Mo	Bt	Bt Mo	Peribronchiolar lymphocytic cuffs and alveolar histiocytosis, otitis media
Yellow circle	62	Bt Pm Mo	Bt Pm	Bt Mo	Leukocytolysis, peribronchiolar lymphocytic cuffs and bronchiolar epithelial hyperplasia, fibrinous pleuritis, fibrinous peritonitis
Yellow 14	63	Bt Mo	X	Bt Mo	Peribronchiolar lymphocytic cuffs, rare lungworm larvae
White X	64	Bt Mhg Mo	Bt	Bt Mo	Peribronchiolar lymphocytic cuffs and bronchiolar epithelial hyperplasia, suppurative bronchitis with intralesional lungworm larvae, necrosuppurative hepatitis
Blue X	72	X	X	X	Otitis media, carcass too autolysed for histopathology
Orange 20	73	Bt Mo	Bt	Bt Mo	Leukocytolysis and peribronchiolar lymphocytic cuffs, otitis media

[a] Bt = leukotoxigenic *Bibersteinia trehalosi*; Mo = *Mycoplasma ovipneumoniae*; Mhg = leukotoxigenic *Mannheimia haemolytica* or *Mannheimia glucosida*; Pm = *Pasteurella multocida*; X = not sampled.

[b] Age was estimated for this uncollared lamb, based on age of a similarly sized collared lamb found on the same date, near the same location.

larvae were infrequently encountered histologically (Table 2).

Seven of the 11 lambs with pneumonia had suppurative otitis media. Bulla swabs routinely yielded both leukotoxigenic *B. trehalosi* and *M. ovipneumoniae* (Table 2). Lambs with pneumonia were variably affected by fibrinous pleuritis (*n*=5), fibrinous peritonitis (*n*=1), and necrotizing to necrosuppurative hepatitis (*n*=3). *Bibersteinia trehalosi* was isolated in all cases when liver tissue was available for culture and PCR (*n*=7), confirming sepsis. In one case, *Pasteurella multocida* also was identified in liver; this was the only case of fibrinous peritonitis similar to previous cases of *P. multocida*–associated fibrinous polyserositis in bighorn lambs (Wood et al. 2017).

We found strong evidence that pneumonia was severely depressing recruitment in the Granite bighorn herd, accounting for death in 69% (11/16) of the lamb carcasses examined. Only one lamb died from predation, suggesting this was not a significant factor contributing to lamb mortality at Granite. Predation was readily detected in South Dakota, US, bighorn herds where lamb losses to both pneumonia (36%) and predation (30%) were documented using field methods nearly identical to ours (Smith et al. 2014a). Lambs killed by predators in the South Dakota herds also were far younger (mean 36 d old) than lambs succumbing to pneumonia (60 d [Smith et al. 2014a] vs. 61 d in our study). Similar vulnerability of younger lambs to predation also was reported in a bighorn herd without respiratory disease (Hass 1989).

We attributed lamb pneumonia in the Granite bighorn herd to mixed infection with respiratory pathogens including *M. ovipneumoniae* and leukotoxigenic *B. trehalosi,* although parainfluenza 3 virus, lungworms, and perhaps other agents also may have contributed. This combination of respiratory pathogens is common to other bighorn herds in Colorado suffering from poor recruitment (e.g., Wolfe et al. 2010; Sirochman et al. 2012; Wood et al. 2017), but other patterns of disease and pathogen profiles have been observed elsewhere (e.g., Besser et al. 2013). It follows that broadly effective tools and management strategies not focused on a single pathogen may be of greatest value in conserving bighorn herds struggling with various manifestations of respiratory disease throughout the western US.

The capture of neonatal bighorn lambs was facilitated by using VITs. However, losing three of the 15 lambs handled in this study to abandonment was troubling. Although the proximate cause of death in these three lambs was starvation, we believe that the most unbiased interpretation of starvation in recently captured lambs was abandonment. The VIT technology was originally developed and successfully used in mule deer (*Odocoileus hemionus*) dams, which routinely leave and return to neonates (Bishop et al. 2011). Bighorn dams accompany lambs continuously and may not be as willing to return after separation (Smith et al. 2014a). Lamb losses attributed to “handling,” “abandonment,” and “starvation” were reported elsewhere (Smith et al. 2014a), suggesting our experience was not unique. Unfortunately, alternative approaches for marking bighorn lambs also have drawbacks (e.g., Smith et al. 2014b). We do not know the precise triggers for abandonments, but we suspect that—similar to the responses observed in dams of lambs killed by predators (Smith et al. 2014a)—disturbance associated with lamb capture and handling was sufficient to compel the dam to leave the immediate vicinity and not return in these cases. To minimize the risk of abandonment, we recommend that lamb captures be carried out quickly and with as few people, little noise, and minimal disturbance to the ewe as possible, and perhaps most importantly that lambs be released within the line of sight of their dams. Wherever feasible, dam-lamb reunions should be confirmed.

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