Management Responses to Pneumonia Outbreaks in three Washington State Bighorn Herds: Lessons Learned and Questions yet Unanswered

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ABSTRACT Pneumonia induced die-offs and subsequent periods of low lamb survival are the greatest impediments to restoration of historic bighorn sheep abundance in North America, and developing effective responses to disease outbreaks in bighorn sheep has been frustrating for wildlife managers. A difficulty in understanding the phenomena is that no 2 situations seem identical. Thus, careful documentation of individual events is needed to understand common patterns and processes. We provide an update on 3 bighorn herds in Washington State that recently experienced pneumonia-related declines (Yakima Canyon, Asotin, and Tieton); management responses and outcomes differed in each case. The Yakima Canyon herd experienced an all-age die-off during winter 2009-2010, during which we culled animals showing signs of respiratory disease. In 2011 and 2012, the herd briefly rebounded, but then suffered 2 consecutive years of recruitment less than 10 lambs:100 ewes, accompanied by pneumonia (cohorts born in 2013-2014). The Yakima Canyon herd is characterized by considerable spatial structuring that was reflected in intra-herd patterns of disease. The Asotin herd suffered an all-age die-off in 2012, and we took no management actions during the outbreak. Similar to Yakima Canyon, after 1 year of high lamb mortality, survival returned to normal and we failed to detect evidence of disease. In 2015 we removed 3 ewes (~10% of survivors) that tested positive for *Mycoplasma ovipneumoniae* and recruitment has been 31– 54 lambs:100 ewes with no pneumonia or *M. ovipneumoniae* detected through the 2015 cohort. The Tieton herd suffered a catastrophic all-age die-off in winter 2013. Due to its proximity to the adjacent Cleman Mountain herd, we lethally removed every Tieton individual that did not die of pneumonia during the outbreak. To date, the Cleman Mountain herd has shown no evidence of pneumonia. While we do not assert that our management response was the only reason for the differing outcomes, we hope that follow-up monitoring and replication will help us identify which actions are effective at controlling the impacts of disease in bighorn sheep.

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KEY WORDS bighorn sheep, culling, disease, mortality, *Mycoplasma ovipneumoniae*, pneumonia, Washington

Bighorn sheep (*Ovis canadensis*) currently occupy a small percentage of their historic distribution in western North America. Although habitat degradation, hunting, disturbance, and other factors have contributed, mortality resulting from epizootic pneumonia is now thought to be the primary cause of the historic decline and remains the primary factor limiting bighorn sheep recovery (TWS 2010, 2014; Wehausen et al. 2011). Our understanding of the etiology and mechanisms of disease transmission has recently undergone considerable advancement and refinement (Dassanayake et al. 2010, Lawrence et al. 2010, Besser et al. 2012). There is now clear evidence that polymicrobial pneumonia epizootics are typically triggered by spillover of *Mycoplasma ovipneumoniae*, a bacterium commonly carried by domestic sheep and goats that can also suffer pneumonia (Da Massa et al. 1992, Nicholas et al 2008, APHIS 2015), but which is sometimes portrayed as of minor importance to sheep producers (Scott 2017). When introduced into bighorn sheep, *M. ovipneumoniae* binds to respiratory cilia, interfering with mucociliary clearance, thus facilitating invasion of the lungs by other species of bacteria, often resulting in fatal pneumonia (Besser et al. 2008, 2012, 2013, 2014).

After initial transmission, pneumonia events can cause up to 100% mortality in a bighorn sheep herd and typically result in depressed lamb survival for many years afterwards (Foreyt and Jessup 1982, Cassirer and Sinclair 2007, Cassirer et al. 2013). Once introduced, the disease can be spread within and among bighorn sheep herds. Managers have struggled to find ways to cleanse herds of pneumonia once introduced, as vaccines and treatments have generally been ineffective, impractical, or both (Foreyt 1992, Miller et al. 1997, Cassirer et al. 2001, Sirochman et al. 2012). Most commonly, no actions are taken because none can be. At the other extreme is depopulation of the entire herd (McAdoo et al. 2010, McFarlane and Aoude 2010). Between these are interventions such as culling, reducing, or cordoning, conducted to reduce the spread or effect of disease. Managers rarely if ever have the luxury (or logistics) of treating such interventions as formal experiments, which would require the presence of a suitable experimental control. Instead, we generally move forward armed only with hypotheses to link our actions with expected consequences, and must assess our success by comparing multiple case histories, each accompanied by its suite of particular circumstances and particularities.

In this paper, we present case histories of 3 bighorn sheep herds in the state of Washington that were affected by pneumonia outbreaks and die-offs, making reference to relevant, nearby herds when appropriate. Although not designed specifically to do so, these retrospective case studies illustrate an array of management approaches which, together with 3 to 6 years of subsequent monitoring, allow us to draw some general lessons. Although the origins of these disease outbreaks are ultimately of interest, we were unable to pin-point any of them. Instead, we focus in this paper on the temporal and spatial course of the disease events, responses on the part of the Washington Department of Fish and Wildlife (WDFW) and partners to stem them or keep them from spreading, and thus encouraging population recovery, and, finally, on inferences we have been able to draw on pneumonia in bighorns generally. We are fortunate in that, in all 3 cases, WDFW and

partners had, prior to the disease event, developed information on the herd, including approximate population size; movements, range use, and spatial sub-structuring (primarily using VHF telemetry); and had conducted pathogen surveillance (i.e., history of previous infection with, and exposure to relevant bacteria).

Bighorn sheep in Washington were completely extirpated by the early 20th century; all existing herds are the result of agency-implemented reintroductions (Johnson 1983, 1996). However, the current geographic distribution of bighorns, although fragmented and reduced in abundance, resembles closely that estimated from both historical accounts and archeological evidence from Native Americans (Lyman 2009). Areas where bighorns historically occurred but have not been reintroduced are typified by human development and occupation.

Study areas

The Yakima Canyon, Tieton, and Cleman herds are located in Southcentral Washington (Fig. 1). The herds are co-managed with the Yakama Nation and Muckleshoot Indian Tribes (MIT). The Asotin herd is in southeast Washington and is co-managed with the Nez Perce Tribe.

The Yakima Canyon herd occupies both sides of the canyon formed by the Yakima River as it flows northward through the low hills known as Umtanum Ridge, between Ellensburg and Selah, WA (Fig. 1). Most of the core range west of the Yakima River is owned by WDFW; lands east of the river are managed by the Bureau of Land Management, Washington Department of Natural Resources (WDNR), The Nature Conservancy, or private owners. Elevations vary from approximately 400m to 1,380m. Vegetation is predominately bunch-grass and sagebrush steppe, with cottonwoods and riparian along the Yakima River and tributaries (WDFW 1995).

The Tieton herd range is located north of the Tieton River, from its confluence with the Naches River (where US highway 12 intersects Washington state highway 410). Land ownership is a mixture of WDFW wildlife areas and Okanogan-Wenatchee National Forest (OWNF). Elevations vary from approximately 500m to 1,500m. Vegetation consists primarily of bunchgrass steppe on open slopes, and mixed conifer in draws. The adjacent Cleman herd occupies primarily public (WDFW and OWNF) lands north of the Naches River (along state highway 410), dominated by Cleman Mountain and spur ridges associated with Manastash Ridge. Elevations vary from approximately 550m to 1,460m. Vegetation is primarily bunchgrass steppe, with mixed conifer in draws.

The Asotin herd core range is located in the southeast corner of Washington, approximately 24 km southwest of Clarkston, WA, in Asotin and Garfield counties. These steep hills are in the extreme northerly portion of the Blue Mountains. WDFW manages the majority of lands used by the herd, with other major land managers being the Umatilla National Forest and the WDNR. Elevations vary from 425m to 3,629m, with the lower elevations being predominantly in private ownership. Vegetation is primarily perennial bunchgrass (*Pseudoroegenaria spicata*), bare rock, and talus, with north slopes containing shrub and open timber at higher elevations (WDFW 1995).

Methods

Population monitoring

All 3 focal herds were surveyed by helicopter and/or ground surveys annually, although timing of surveys differed. Abundances were derived from minimum counts and mark-resight estimates that we suspect typically tracked true abundance closely. In Asotin we monitored summer survival of lambs born to marked ewes each

year. In Tieton and Umtanum, we estimated lamb:ewe ratios in September (from which we inferred magnitudes of summer lamb mortality). We used lamb:ewe ratios in March or April as an index of recruitment to the yearling age-class. We note that when estimating lamb:ewe ratios, "ewe" includes yearling females, which we rarely could differentiate from older adult females, and which were too young to reproduce in that year. Abundance of the Cleman bighorn herd, which was not affected by pneumonia but is adjacent to the Tieton herd (Fig. 1), was estimated by ground counts in mid-winter at a feeding station.

All 3 herds (as well as the adjacent Cleman herd) had been subject to some level of radio-telemetry monitoring. Radio-collared ewes in the Asotin herd were monitored weekly from the ground during spring through autumn, and biweekly during late autumn and winter. We also conducted weekly fixed-wing telemetry flights to locate animals missed during ground surveys in spring and monthly during the rest of the year. The Yakima Canyon, Cleman, and Tieton herds have been monitored via both VHF and GPS collars.

Health testing

All sheep populations had some level of health monitoring prior to the outbreak. The Asotin herd is part of the Hells Canyon metapopulation, which has been involved in collaborative research by the consortium of states (Idaho, Oregon, Washington) since 1997, and as such, was monitored most intensively. Bighorn sheep were captured for health sampling in all herds, primarily by helicopter net gunning. Some animals were captured via ground darting with immobilization agents in the Asotin herd. Aerial captures occurred during the winter months of November through February. Ground captures occurred September through December. In the Umtanum population we also collected samples (e.g., whole carcass, nasal swab, blood, lungs) for *M. ovipneumoniae* opportunistically from hunters 2010-2015, and from dead, dying, or selectively culled lambs 2013–2015.

Live animal biomedical sampling included collection of oropharyngeal swabs kept cool and submitted within 24 hours for aerobic bacterial culture for Pasteurellaceae and other aerobic bacteria. Nasal swabs were submitted for culture and polymerase chain reaction (PCR) for *M. ovipneumoniae.* Nasal washes of 50 ml buffered saline were collected in the Umtanum population in 2013. Blood serum was collected and submitted for competitive enzyme-linked immunosorbent assay (cELISA) to test for antibodies to *M. ovipneumoniae*. Intact carcasses from animals that were culled in the Tieton and Yakima Canyon populations, or found recently deceased, were additionally subjected to routine necropsy, including histology; as well as sampled from one or more of the following anatomical sites for *M. ovipneumoniae* PCR: nose, nasal sinuses, tonsils, tympanic bullae, trachea, tracheobronchial lymph nodes, and lungs. Samples available from hunter-harvested rams were generally limited to nasal swabs, blood samples, and lung tissue. All diagnostic tests were completed at the Washington Animal Disease Diagnostic Laboratory (WADDL) at Washington State University, Pullman.

Strain-typing of *M. ovipneumoniae* (Cassirer et al. 2016) was conducted in the T. E. Besser laboratory at Washington State University (WSU), as was PCR testing for *M. ovipneumoniae* on nasal washes.

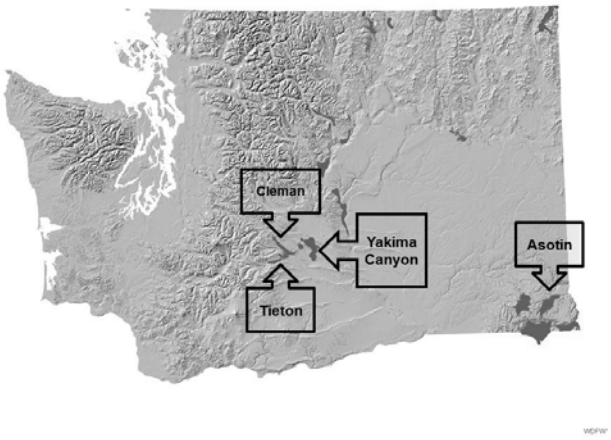

Figure 1. Topographic image of Washington state, showing core ranges of bighorn sheep herds (solid polygons), with locations of the 4 herds discussed in this paper (Yakima Canyon, Asotin, Tieton, Cleman) indicated by arrow boxes.

Results

Yakima Canyon herd

Herd origin and pre-outbreak population performance: The Yakima Canyon herd was established in 1970 with the release of eight animals in what we subsequently began terming Umtanum North (Fig. 2). These 8 founders were translocated from the nearby Quilomene herd in Washington (these animals, in turn, originated from a translocation of animals from Williams Lake, B.C. in 1964). Within 15 years, the Yakima Canyon population had grown to an estimated 200 animals, and some individuals had moved easterly across the Yakima River to form the Selah Butte sub-herd. However, no natural colonization occurred in the southern portions of the canyon. In 1996, WDFW captured 14 individuals in Selah Butte North and Central sub-herds, and moved them to what subsequently grew to become the Umtanum South sub-herd. Only a few of these 14 became resident; thus, in 2001, an additional 11 bighorns were added (in this case, from the distantly-located Lincoln Cliffs herd). This Umtanum South sub-herd grew rapidly and subsequently crossed the Yakima River to form the Selah Butte South sub-herd. By 2006, over 300 animals occupied the canyon, comprising 5 sub-herds on both sides of the Yakima River, and it remained at about approximately this level until 2009 (WDFW 2016). Serology on 11 animals tested in 2007 indicated that the Yakima Canyon herd had not been exposed to *M. ovipneumoniae*, at least within the 2 years prior to the outbreak of 2009-2010 (Fig. 3).

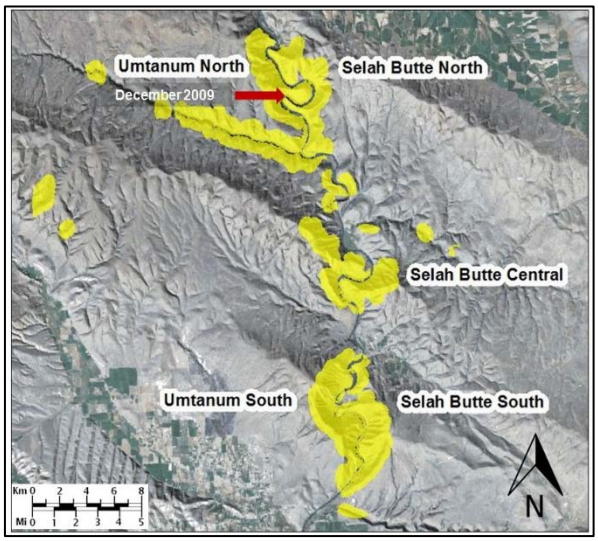

Figure 2. Spatial structure of the Yakima Canyon bighorn herd, Washington, USA (shown in yellow shading), illustrating the sub-herds (from north to south) Umtanum North, Selah Butte North, Selah Butte Central, Umtanum South, and Selah Butte South. The Yakima River runs north to south, separating the Umtanum side (to the west) from the Selah Butte side (to the east). Red arrow shows approximate location of the initial detection of the pneumonia outbreak in December 2009. Despite the presence of mapped habitat across the river directly west of the Selah Butte Central, bighorn ewes used this area sparingly, and thus it is not named as a subherd.

Outbreak and short-term management response: During winter 2009-2010, a poly-microbial pneumonia outbreak caused the loss of approximately 13% of the herd. When

initially detected in November 2009 (and confirmed by WDFW in December 2009), the outbreak was thought to be isolated to the Umtanum sub-herd. Forty-three bighorns were known to have died (presumably from pneumonia), all but 1 in the northern portion of the Umtanum sub-herd. In December 2009, we euthanized 8 animals: 5 on the west side and 3 from the east side, and submitted them to WADDL to document and confirm the cause of the outbreak. Three sheep on the west side were pneumonic, and all were *M. ovipneumoniae* positive. All 3 sheep on the east side were healthy. In February 2010, we began selectively culling sheep on the west side with the most severe clinical signs, with the goal of removing individuals thought likely to be shedding bacteria from the population. The hypotheses undergirding this symptom-based culling were that doing so would stop the infection from crossing the river eastward (from the Umtanum to Selah Butte sub-herds), and increase lamb survival in subsequent cohorts. Criteria for culling were visual evidence of clinical disease, defined as coughing, nasal discharge, droopy ears, and/or lethargy. Most culling was conducted on contract with the U.S. Department of Agriculture, Wildlife Services Division. We lethally removed 52 symptomatic and 6 asymptomatic animals west of the river, and another 3 asymptomatic animals east of the river. In addition, samples were collected from 4 fresh carcasses encountered west of the river during the course of live animal removals (Table 1; an additional 43 carcasses were found west of the river, but were too decomposed to allow sampling).

Serologic testing confirmed exposure to *M. ovipneumoniae* in 98% of 53 symptomatic sheep removed from Umtanum (Fig. 3). Most (97%) sheep sampled in Umtanum were also PCR positive for *M. ovipneumoniae* in lung tissue or on nasal swabs (Fig. 4), and 77% had histological evidence of pneumonia. All 6 non-symptomatic sheep sampled in Umtanum in March 2010 were PCR and seropositive for *M. ovipneumoniae*, but only 3 had histological evidence of bronchopneumonia. A single strain of *M. ovipneumoniae* was identified in all samples. Among the 6 euthanized in Selah Butte during the outbreak in Umtanum, none were seropositive or PCR positive for *M. ovipneumoniae*, but 1 had histological evidence of bronchopneumonia. Culturing of swab and lung samples collected from 53 symptomatic animals during the outbreak revealed presence of *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mannheimia haemolytica*, (Table 2). *Moraxella* sp. (49% of samples), *Arcanobacterium pyogenes* (40% of samples); a single case of *Staphylococcus aereus* were also cultured.

Post-outbreak. Lamb recruitment (Table 2) was low in 2010 but rebounded to pre-outbreak levels in 2011 and 2012 (Fig. 5), and by spring 2012, the estimated population had increased to near pre-2009 levels. In 2013, initial lamb production was high, but by early September all lambs of marked ewes in Umtanum had died and the overall lamb:ewe ratio was 12:100 (and had declined to 8:100 by March 2014; Table 2). Two lambs collected west of the river had bronchopneumonia and were PCR positive for the same strain of *M. ovipneumoniae* documented in 2009-2010. Lambs east of the river survived longer, but by early winter 2014, most of these had also died. In 2014, overall lamb recruitment was similar, with the lamb:ewe ratio declining to 8:100 by winter 2014-15 and pneumonia was confirmed at WADDL. Over-winter recruitment of the 2015 lamb cohort (to March 2016) recovered somewhat from the low of the previous 2 years, to 23:100 overall (Table 3) but was highly variable by sub-herd (see below).

Table 1. Detection of *Mycoplasma ovipneumoniae* via PCR in asymptomatic, symptomatic and dead bighorn sheep in the Yakima Canyon herd during the 2009-2010 pneumonia outbreak. Rows represent animal condition at the time of sampling.

	Detected	Indeterminate	Not Detected
Symptomatic (culled)	51	1	0
Early Umtanum sampling	5[a]	0	0
Selah Butte visually healthy	0	0	3[b]
Found dead	3	0	1[c]

[a] One was symptomatic when sampled. All animals are from the Umtanum sub-population. Three had pneumonia lesions in lungs at necropsy.

[b] One had histological evidence of pneumonia.

[c] Lung tissue from this animal was not available for testing, PCR on nasal sinus sample was negative.

Table 2. Prevalence of 3 genera of Pasteurellaceae bacteria (% of sampled adults), as detected by culture before, during, and after pneumonia outbreaks in 3 bighorn sheep populations, 2008-2016, Washington State, USA. *P. t. = Bibersteinia trehalosi; P.m. = Pasteurella multocida; M. = Mannheimia spp.*

Sample	Yakima Canyon			Asotin			Tieton		
	B.t.	*P.m.*	*M. h.*	*P. t.*	*P.m.*	*M. h.*	*P. t.*	*P.m.*	*M. h.*
Pre-outbreak				100		17	----77[a] ----		
During outbreak	45[b]	18	4	100	33	50	71[c]	13	13
6 months post outbreak	63[d]	0	25	19		69			
2 years post outbreak				88[e]	0	16			
3 years post outbreak	84[f]	0	32	40	0	0			

[a] *n* = 22, Pasteurellaceae aggregated, none detected in 5 animals.

[b] *n* = 51, includes found dead, culled, and euthanized with no symptoms, none detected in 17 animals.

[c] *n* = 24, includes found dead and culled, none detected in 4 animals.

[d] *n* = 8, hunter sampled animals; none detected in 2 animals.

[e] *n* = 32; 9% undifferentiated *Pasteurella* spp.

[f] Live capture, 25 ewes, 6 rams (*n* = 31), none detected in 2 animals

Table 3. Summary of over-winter lamb recruitment in herds affected by all-age pneumonia die-offs in Washington State, 2009-2016. Years represent spring following disease event (e.g., Year 1 = 2010 for Yakima Canyon, 2013 for Asotin). Shown are sheep:ewe ratios (ewes defined as females older than lamb).

Herd	Yakima Canyon	Asotin
Management response	Removal of symptomatic animals during outbreak	Removal of PCR positive ewes 2 - 3 years after outbreak
	Mean lamb recruitment to March of year following event	
Year 1	10:100	9:100
Year 2	58:100	31:100
Year 3	42:100	52:100
Year 4	8:100	50:100
Year 5	8:100	-
Year 6	23:100	-

Table 4. Attributes of herds affected by pneumonia die-offs, Washington State, 2009-2013. Single quotes denote that we do not necessarily accept the scientific validity of these names as formal sub-species, but rather manage them separately pending clarity about the biological basis for doing so (Wehausen and Ramey 2000).

Herd attributes	Yakima Canyon	Asotin	Tieton
Bighorn Ecotype	'California'	'Rocky Mtn'	'California'
Year of Origin	1970	1973	1998
Subsequent augmentations	1996, 2001	1991, 1993, 1994, 1998	1999, 2000, 2001,2003
Year of outbreak	2009-2010	2012	2013
Estimated abundance at outbreak	> 300	101	200
Estimated herd decline	35%	36%	> 50% prior to complete removal
Management response	Culling symptomatic animals	Removal of putative super-shedders	Complete herd removal
Current status	Declining slowly, but sub-herd performance variable	Evidently slowly increasing	Gone; a few wandering animals from adjacent Cleman herd
Tentative assessment	Failed to stop spread of disease or disease persistence	Population is healthy 4 years after outbreak	Herd extirpated, but adjacent Cleman herd still free of pneumonia

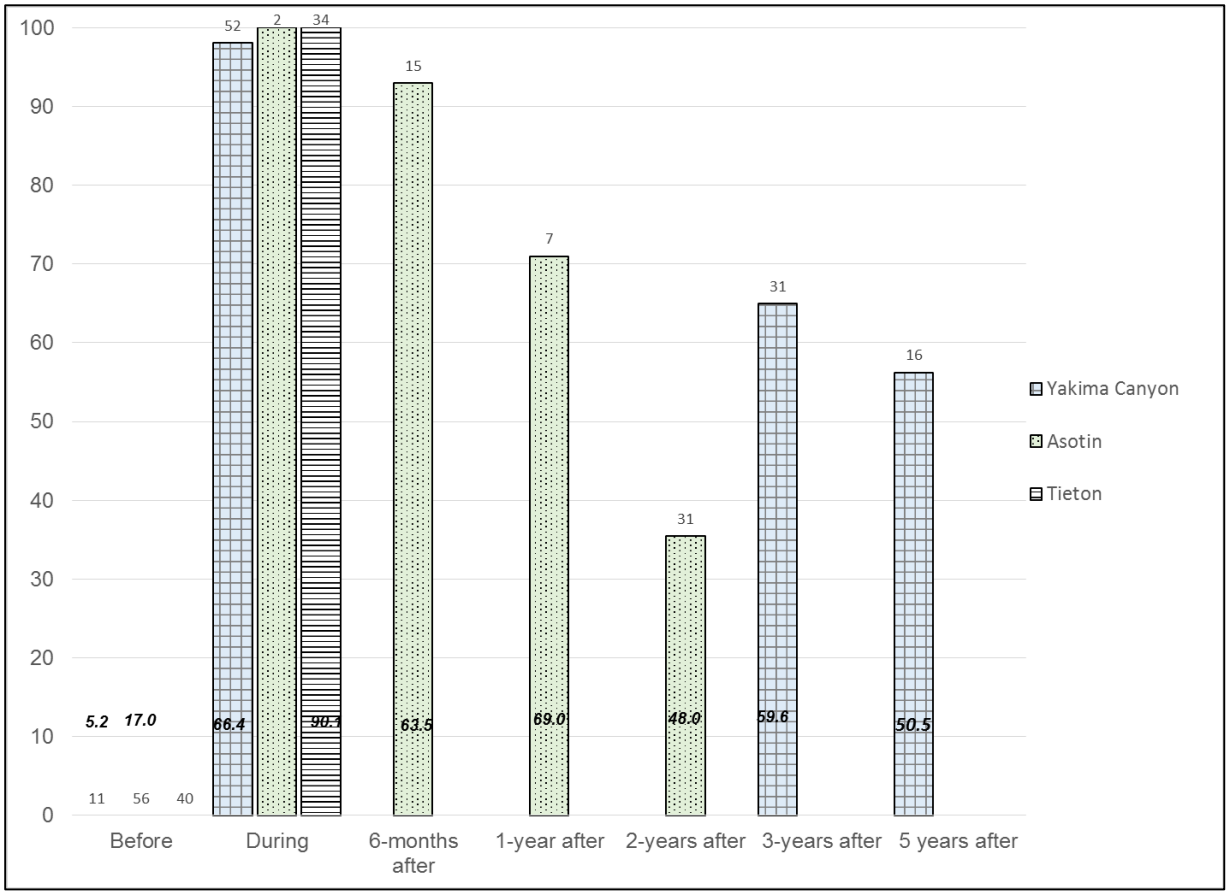

Figure 3. Evidence of exposure to *M. ovipneumoniae* in sampled animals, before, during, and after pneumonia outbreaks in 3 bighorn sheep populations, 2008-2016, Washington State, as indicated by ELISA serology. Shown are percentages of animals testing positive (defined as above the threshold of 40). Where available, mean titers are shown in bars. Sample sizes for each test appear above bars.

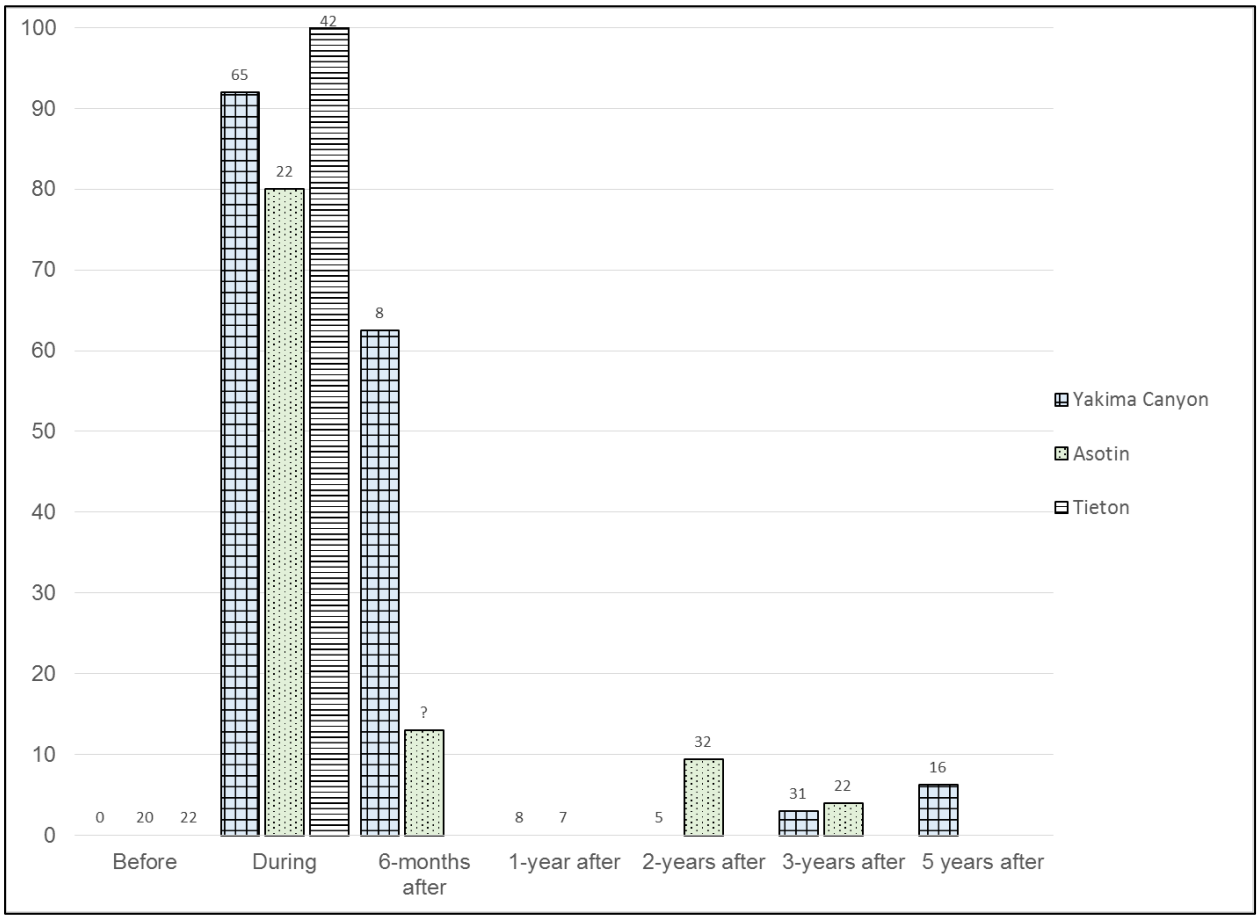

Figure 4. Evidence of *M. ovipneumoniae* infection in sampled animals, before, during, and after pneumonia outbreaks in 3 bighorn sheep populations, 2008-2016, Washington State, as indicated by PCR from various tissues. Shown are percentages of animals testing positive. Sample sizes for each test appear above bars.

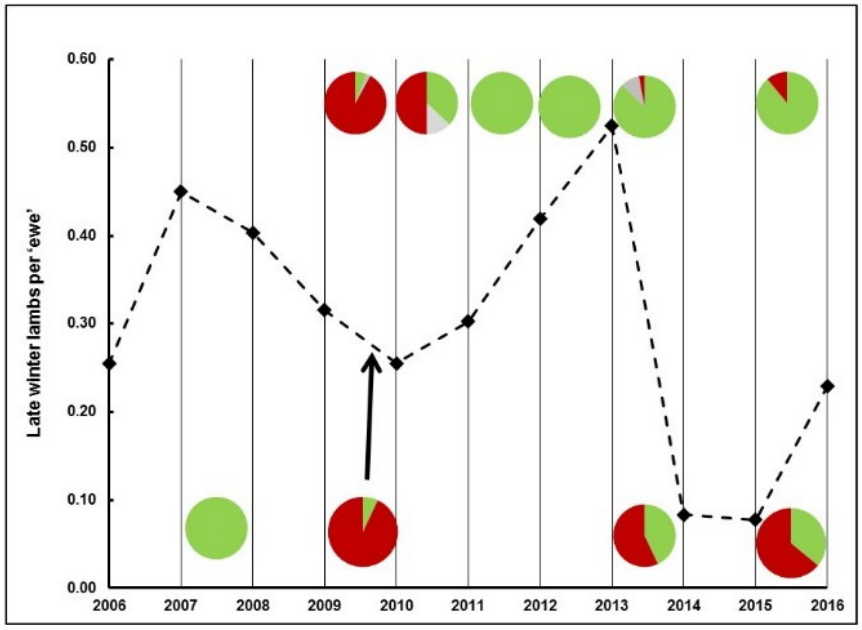

Figure 5. Late winter lamb:ewe ratios of the Yakima Canyon bighorn sheep herd, Washington State, 2006-2016 (dashed line). Pie graphs above show the proportion of individuals tested positive for active *Mycoplasma ovipneumoniae* using PCR from nasal swabs. Pie graphs below show the proportion of individuals with positive ELISA serology titers for *M. ovipneumoniae*. Red = positive; green = negative, grey = indeterminate.

In autumn 2010, 4 animals taken by hunters from Selah Butte were positive for *M. ovipneumoniae* in respiratory tracts on PCR, and all 4 also had moderate to severe bronchopneumonia. Of 4 additional hunter-harvested animals taken from the Umtanum sub-herd, 1 animal was *M. ovipneumoniae*-positive in the respiratory tract, but none of the 4 had bronchopneumonia. However, sampling of bronchial and/or lung tissue from 11 hunter-killed samples in 2011 and 2012 failed to detect *M. ovipneumoniae* (Fig. 4).

In February 2013, 65% of 31 bighorn sheep sampled were positive for serologic antibodies to *M. ovipneumoniae* (Fig. 3). In the Umtanum sub-herd, 71% were positive, whereas 50% were positive in the Selah Butte sub-herd. Nasal swabs from 3 animals were indeterminate on PCR for *M. ovipneumoniae.* A nasal wash sample from one of these individuals, an Umtanum ram, was PCR-positive (Fig. 4).

In February 2015, we captured and tested 17 ewes, including recapture of 15 of the ewes we had sampled in 2013. Nine of 16 (56%) were seropositive for exposure to *M. ovipneumoniae* (3 were indeterminate). Serum antibody percent inhibition (%I) increased in ewes that were seronegative in 2013 ($\bar{x}$ = 23%I in 2013, $\bar{x}$ =46 % I in 2015) and decreased in animals that were seropositive in 2013($\bar{x}$ = 80%I in 2013, $\bar{x}$ 67% I in 2015). Two ewes were PCR-positive: A nasal swab from a Selah Butte sub-herd ewe was positive, and an Umtanum sub-herd ewe that died as a result of capture was PCR-negative on nasal swab but PCR-positive on lung tissue. The ram that had tested PCR positive for *M. ovipneumoniae* in 2013 was no longer living at the time of the 2015 capture. Neither of the 2 animals that tested positive in February 2015 had done so in February 2013, and the 2 ewes that tested indeterminate in 2013 were negative in 2015.

Asotin herd

Herd origin and pre-outbreak population performance: The Asotin Creek bighorn sheep population originated with the release of 4 animals from the nearby Tucannon Herd in 1973. These animals were augmented by animals from the Hall Mountain herd near Sullivan Lake in northeastern Washington (6 in 1991, and another 9 in 1994), and from Spence's Bridge, British Columbia (10 in 1997). From 2004 to 2010, the population grew at a mean annual rate of approximately 18% despite removal by

WDFW due to concerns about contact with domestic sheep and goats (~20 females and ~10 males), for translocation, for use in captive disease experiments primarily at Washington State University, and by licensed hunters. March lamb:ewe ratios averaged 41:100 (approximate 95% confidence interval 34-47:100). The largest recorded population size (in 2011, prior to the outbreak) was approximately 105 sheep. Post-outbreak population estimates in 2012 and 2013 were 70 animals, declining to 60 in 2014. Prior to the outbreak, we had documented no evidence of pneumonia or *M. ovipneumoniae (*health history included blood samples collected in captures for removals and radio-collaring in 2006, 2008, 2009, and 2011, nasal samples collected 2008 – 2011, and necropsies 1997 – 2011).

Outbreak and short-term management response: In January 2012, both contagious ecthyma (orf, not previously documented in the Asotin population), and pneumonia associated with infection with *M. ovipneumoniae* were confirmed by WADDL, and by April 2012, 30% of the ewes had died. Orf was also observed in adjacent bighorn sheep populations in the fall of 2011. One half curl ram captured in the town of Asotin was observed with orf lesions, but no other disease was evident in rams, and ram survival appeared high. Initially, no management actions were taken in response to the outbreak, because other bighorn populations to the south were already known to be infected and the *M. ovipneumoniae* strain in Asotin (IGS 404) matched the predominant strain in the Hells Canyon bighorn sheep metapopulation since 1995 (Cassirer et al. 2016). Four of the 5 mortalities submitted to WADDL during the early 2012 outbreak were positive for *M. ovipneumoniae* on PCR in lung tissue (Fig. 4). Serology conducted on one ewe that had orf but not pneumonia indicated she was negative for antibodies (Fig. 3).

Post-outbreak: In May 2012, at least 14 lambs were observed born to the approximately 25 ewes that survived the outbreak. On June 8, 2012, at approximately 3 weeks of age, 2 lambs were collected by gunshot and necropsied. Both lambs were healthy and uninfected with *M. ovipneumoniae*, but had serologic antibody titers, probably passively-transferred from their dams, indicating the adults had been exposed during the outbreak. As a control, 2 lambs similarly collected in the Black Butte bighorn population approximately 30 km south at Heller Bar on June 1 were pneumonic on necropsy and infected with *M. ovipneumoniae*. Serologic titers were similar in both sets of lambs. However, by March 2013, only 5 lambs were documented from 32 surviving Asotin ewes (Table 3). Lamb recruitment improved in 2013 to just under pre-outbreak levels (9 lambs surviving to spring 2014 from 28 ewes, ratio 31:100; Table 3). In summer 2014, lamb survival was 88%, and subsequent recruitment (to early winter 2015) was 13 lambs from 25 ewes (52:100; Table 3), slightly higher than the pre-outbreak mean. Similarly, the 2015 cohort as of early winter 2016 was 16 lambs from 32 ewes (50:100; WDFW 2016).

By October 2012, PCR detection of *M. ovipneumoniae* infection had fallen to 13% (Fig. 4), although 93% retained antibodies(Fig. 3) including 6 of 7 rams, indicating that they had been exposed although all were PCR negative. One ram taken by a hunter in September 2012 was healthy but PCR positive in the lungs and nose and seropositive. PCR detection of *M. ovipneumoniae* in adults remained between 11 and 20% through 2014.

During November 2013 - February 2014, prevalence of *M. ovipneumoniae* on nasal swabs was 22% in 9 lambs, 0 of 2 yearlings, and 11% in adults (n = 19). Antibody titers to *M. ovipneumoniae* were detected in 33% of lambs and 84% of adults. PCR prevalence for animals captured September 2014 – January 2015

was 20% for adults (n = 15). All lambs and yearlings tested were negative on PCR and ELISA, including a lamb that had been positive in February 2014. Antibodies were detected in 67% of adults (Fortin and Cassirer 2015; Fig. 3).

In 2013, the 3 states involved in the Hells Canyon Initiative agreed to a program of experimental management for the Asotin Herd (as well as the nearby Black Butte herd in Washington, and the Lostine herd in Oregon), in which the "super-shedder" hypothesis would be tested (Lloyd-Smith et al. 2005, Matthews et al. 2006). Briefly, preliminary data gathered from both field and captive settings had suggested that bighorns were individualistic and highly heterogeneous in their propensity to become carriers --and thus transmitters -- of *M. ovipneumoniae* (Cassirer 2014, Fortin and Cassirer 2015). Even in populations in which the pathogen persisted over a number of years, it appeared that a small number of chronic carriers, or "super-shedders" might be responsible for a disproportionate amount of vertical and lateral transmission. If so, and if these "super-shedders" could be identified and removed, it might greatly decrease the time required for the pathogen to become extinguished within the population. Thus, a more intensive program of repeated capture and testing of Asotin female sheep was initiated in autumn 2013. Ewes that tested PCR-positive from nasal swabs for *M. ovipneumoniae* repeatedly, i.e., super-shedders, were captured and transferred to a captive facility at South Dakota State University, where a companion study was being conducted. In January 2015, 3 ewes identified as chronic shedders were removed from the population.

Between 19 February and 7 April, 2015, 4 radio-collared, *M. ovipneumoniae*-negative adult ewes in the Asotin population were killed by cougars. The timing and proximity of the kills suggested predation by a single cougar. Because this represented >15% of the ewes in this heavily studied experimental population, we used a local houndsman to assist in removing the cougar. An adult female cougar was removed after the 4th bighorn sheep mortality on April 8, and no further deaths attributable to predation were detected. However, including the cougar predation, 22 adults died or were removed from the population in 2014 and 2015. Five ewes and 2 rams were transferred to the captive facility at South Dakota State University as part of the super-shedder experiment, and 1ewe died during capture. At least 8 rams were harvested by hunters, a 9th was poached but not recovered, and 1 yearling ram was removed from the town of Asotin. Altogether, these known non-pneumonia-related losses slowed recovery of this small population after the outbreak (WDFW unpubl. data, Fortin and Cassirer 2015).

Tieton herd

Herd origin and pre-outbreak population performance: Bighorn sheep were reintroduced to the Tieton area in 1998–2002 with the translocation of 54 sheep from 4 separate herds. Population growth was rapid, particularly after 2004, and by early 2012 had stabilized at approximately 200 animals despite the legal harvest of 63 animals (during hunting seasons 2004-2012) as well as the removal of 72 for translocation. Based on modeled habitat and home range (O'Brien et al. 2014) from GPS collars, the density estimate was 6.2 sheep per km^2. Twenty-two animals used for translocation were tested for the presence of *M. ovipneumoniae* in nasal mucosae using PCR (Fig. 4), and serology for exposure was examined on 18 (Fig. 3). No evidence of either infection or exposure was documented.

Outbreak and short-term management response: In early mid-March 2013, we became aware of an unusual cluster of mortalities in the Tieton herd. Veterinary testing of samples from 5 carcasses (5 heads, 4 lungs) found during the initial surveillance

showed that all had bronchopneumonia (either on gross inspection of lungs, histological examination of lungs, or gross inspection of sinuses and/or bullae), evidence of upper and/or lower respiratory tract disease typical of *M. ovipneumoniae* infections, and all 5 tested PCR positive for infection with *M. ovipneumoniae*. Ground and aerial surveys on March 26, 2013 documented only 35 live animals, and approximately as many carcasses. We speculate that disease had entered the herd at least 3 months earlier.

Because of the apparently high mortality of this new outbreak (estimated >50% mortality at first detection) and the herd's close proximity to the yet-uninfected Cleman herd, we elected to euthanize all known Tieton herd survivors. We began by using WDFW staff to lethally remove 10 animals closest to the Cleman herd on April 3, 2013 (an additional animal died from the disease that day). This was followed by contracting with USDA Wildlife Services, which removed an additional 31 animals by helicopter shooting during April 9-12, 2013. One of our tribal partners issued permits for tribal hunters in early April, but only 1 animal was harvested. An additional 8 animals were removed by WDFW in May 2013. WDFW consistently documented only 3 remaining animals, mainly via trail cameras baited with salt. The final 3 were removed by skilled citizens operating under contract from with WDFW in early October 2013. No animals were seen or reported for the remainder of the year. We believe we successfully removed every animal in the herd.

During the outbreak, all sampled animals had severe bronchopneumonia (n = 41) and all were *M. ovipneumoniae* positive on serology (Fig. 3) and PCR (Fig. 4). The final 3 animals removed in October (6 months after we confirmed the outbreak) were all *M. ovipneumoniae* positive on serology and 2 were PCR positive in lungs/cranial bronchus. All 37 *M. ovipneumoniae* identified by PCR from Tieton animals were of a single strain (intergenic spacer [IGS] 388). This strain differed from the single strain that earlier affected the Yakima Canyon herd, and it clustered with domestic sheep rather than goats (T. Besser, pers. comm., 2013). *B. trehalosi* was the most commonly cultured of the Pasteurellaceae bacteria; *P. multocida* and *M. haemolytica* were present less frequently (Table 2).

In April 2013, a domestic wether (castrated male sheep) was found wandering about 10 km from core Tieton bighorn range. A USFS lessee had lost 8 animals the previous fall, believed the sheep to be a survivor, and euthanized the animal. A volunteer collected samples a few days later and WDFW submitted them for testing. Although *M. ovipneumoniae* was detected on PCR from nasal mucosae of this sheep, the strain did not match the IGS 388 strain found among all tested Tieton bighorns. The carcass of another domestic sheep was later discovered within the Tieton herd core range, but was too decomposed to provide samples. WDFW also conducted nasal swabbing for *M. ovipneumoniae* PCR on 10 of 11 domestic, mixed-breed goats held on private land near the Tieton herd. Seven goats tested positive, one negative, and 2 indeterminate for *M. ovipneumoniae*. As with the domestic sheep wether however, neither of the strains (IGS 393 and IGS 423) from these goats matched the IGS 388 strain implicated in the Tieton die-off.

Post-outbreak: We have continued to monitor the Tieton area for bighorns, and have identified none believed to be survivors of the die-off or the subsequent lethal removal. We have received occasional reports from the public of bighorns near the Tieton range, but we believe all resulted from short-distance forays of animals associated with the Cleman herd. Because risks of a new infection had not yet been satisfactorily reduced, WDFW made attempts to remove these animals lethally, but

none were successful. All samples (n = 59) from the Cleman herd have been *M. ovipneumoniae* negative via serology and PCR since fall 2013. Lamb recruitment in the Cleman herd has averaged 40 lambs per 100 ewes (n = 95 lambs) in January.

Herd substructure: Yakima Canyon as a case study

Thus far, we have described demographics and epidemiology for each herd considered as single units. However, it has long been recognized, and recently shown relevant for disease transmission by Manlove et al. (2014), that spatial-social structure characterizes many bighorn herds. In some cases structure is geographically obvious; in other cases, it may be latent and require detailed telemetry, genetic, or epidemiological data to discern. Here, we provide additional detail on demographic and pathogen dynamics on the sub-herds we recognized within the Yakima Canyon herd.

Figure 2 shows the spatial configuration of 5 sub-herds within Yakima Canyon, using the definition of summer bighorn habitat developed by the Hells Canyon project and Payette National Forest (O'Brien et al. 2014) to identify geographic areas. The Yakima River, which flows roughly north to south in this stretch, separates the 2 named "Umtanum" to the west, from the 3 named "Selah Butte" to the east. We are unable to confidently quantify the frequency of interaction among individuals associated with each sub-herd. Our radio-collaring was not designed to estimate frequency of forays. Radio-tracking of VHF collared ewes was sporadic. Only 5 rams wore GPS collars and one of those disappeared before the rut. One of the 2 surviving Umtanum rams moved between sub-herds during the rut (Fig. 6), but died in late winter. The 2 Selah Butte rams were collared in different "sub-herds," but showed almost identical home ranges, covering both sub-herds (Fig. 6). No animals were radioed in Selah south. We first confirmed a radio-marked ewe crossing from the Umtanum to Selah sides of the river in late summer 2016. None of the 3 surviving rams crossed the river. Observations, as well as inference from epidemiology (see below) suggest that river crossing occurs on occasion.

The management intervention in winter 2009-10 was designed to test the hypothesis that symptomatic culling would prevent the infection, which evidently began in the Umtanum North sub-herd but quickly spread to the Umtanum South herd, from spreading eastward across the Yakima River to the Selah Butte sub-herds. However, we documented poor lamb recruitment on both sides of the river as early as spring 2010 (August 2010 lamb:ewe ratios of 4:100 west of the Yakima River and 15:100 east of the river), as well as histological evidence of moderate to severe pneumonia in 4 hunter-killed rams east of the river in autumn 2010. Strain typing confirmed that these 4 rams were infected with the same *M. ovipneumoniae* strain that culling had been intended to halt (Tom Besser, WSU, personal communication). Two of the 4 rams harvested on the west (more heavily affected in the outbreak) side of the river in fall 2010 were *M. ovipneumoniae* negative and none had histological evidence of pneumonia, suggesting that acute infection and disease had subsided by September 2010.

In 2011, lamb recruitment was high west of the river (53 lambs:100 ewes), but only 19:100 east of the river. All of the 3 eastern and 4 western hunter-harvested rams were *M. ovipneumoniae* negative from tissue sampling. In autumn 2012, *M. ovipneumoniae* was not detected among 5 harvested rams, but 1 of the 2 eastern rams had histological evidence of pneumonia. These data were consistent with a continued lag in the epidemic on the east side of the river.

Evidence that disease dynamics continued to vary by sub-herd comes from examining the lamb:ewe ratios during years 4

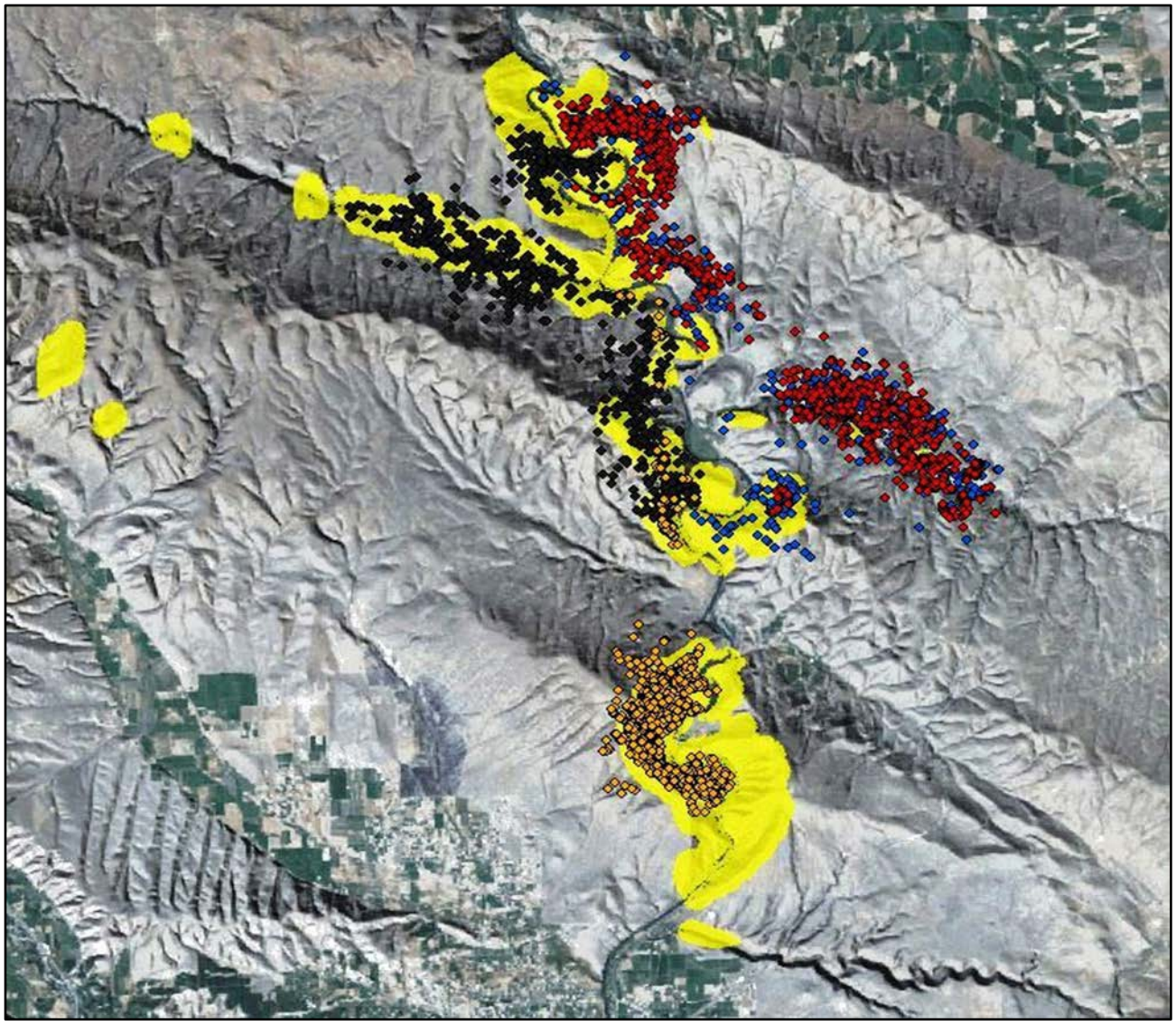

Figure 6. Locations of 4 GPS-radio collared ram bighorn sheep, February 2013-December 2015, within the Yakima Canyon herd, Washington State, USA. Sub-herds (yellow polygons) are as in Figure 2. Each ram is designated with a unique color. The 2 rams on the east (Selah Butte) side travelled among both the Selah Butte North and Selah Butte Central sub-herds; the 2 on the Umtanum side stayed primarily in the sub-herd in which they were collared, but overlapped in habitat between the sub-herd cores. None of the 4 rams was documented to cross the Yakima River, but anecdotal observations indicate that crossings do occur.

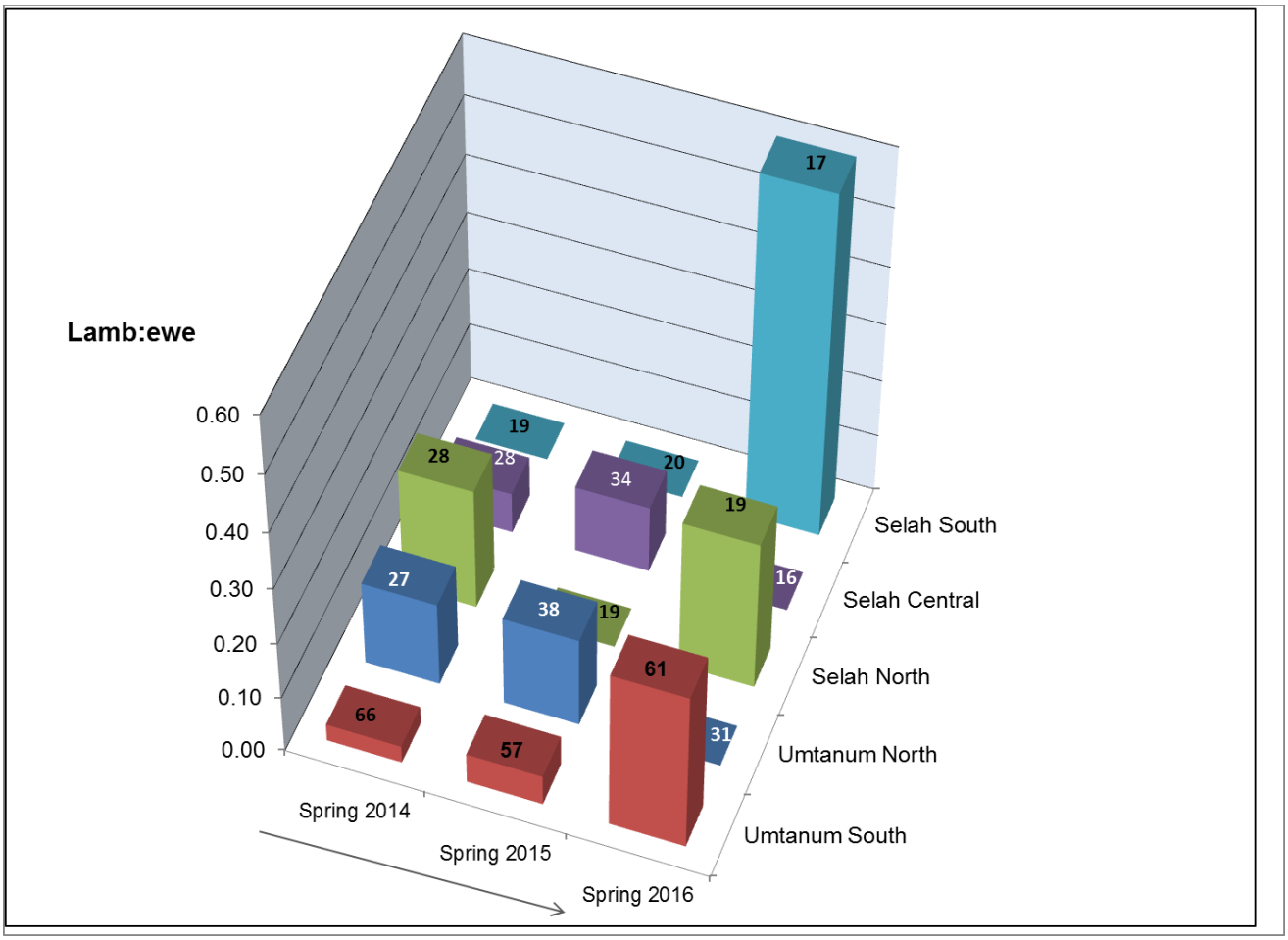

Figure 7. Ratios of lambs to ewes (females older than lamb) recruited to March 2014, 2015, and 2016, for each of the identified sub-herds of the Yakima Canyon bighorn sheep herd, Washington, USA. Numbers atop each bar are number of ewes counted in each sub-herd.

through 6 post-outbreak (cohorts born in 2013 through 2015) in more detail (Fig. 7). The mean Yakima Canyon herd lamb:ewe ratio of 8:100 of March 2014 (i.e., describing recruitment of the spring 2013 lamb cohort) varied from as high as 21:100 (in the Selah North sub-herd) to as low as 0:100 (in in Selah South sub-herd). By March 2015, the overall lamb:ewe ratio was again 8:100, but this similar mean masked a very different geographic pattern: Selah North, with the best recruitment in 2014, had joined Selah South in recruiting no lambs, and most of the limited recruitment came from Selah Central and Umtanum North. In March 2016, overall recruitment had increased somewhat (to 23:100), but had plummeted to zero in the very sub-herds (Selah Central and Umtanum North) providing some recruitment the previous year.

Discussion

Yakima Canyon herd

Based on the movement of pathogens and disease among sub-herds and the continued mortality of lambs after 2010, our management intervention — selectively euthanizing symptomatic individuals — in the Yakima Canyon herd evidently was ineffective in stopping the spread of *M. ovipneumoniae* (Table 4). Based on lamb mortality, symptomatic sheep, and hunter harvested rams, we believe that *M. ovipneumoniae* spread into Selah Butte by June or July, 2010. This would suggest that 1 or more animals moved eastward across the river before that time. Having crossed the Yakima River, subsequent mortality was mostly limited to lambs, and lamb recruitment in 2011 was higher east of the river than where symptomatic animals had been removed.

It is clear to us that visually identifying the animals that were shedding bacteria based on clinical signs was not possible. Even identifying sick sheep was problematic: we found no histological evidence in 20% of the animals euthanized in the South Umtanum sub-herd based on their displaying symptoms, whereas one animal from east of the Yakima River that had pneumonic lungs was *M. ovipneumoniae* negative on both PCR and serology. Our assessment is similar to the conclusions reached by Ramsey et al. (2017) that selective culling failed to contain or lessen pneumonia outbreaks in the East Fork Bitterroot or Bonner herds in western Montana (see also Edwards et al. 2010). Spatially-limited culling of animals during an outbreak in the Black Butte, Washington population in Hells Canyon was also unsuccessful at stopping disease spread (Cassirer et al 1996).

The lamb crop born in spring 2011 (13-15 months post culling) in the 2 sub-herds on the west side of the river had high survival, although *M. ovipneumoniae* was likely still present. East of the Yakima River, where no selective removals took place, lamb recruitment in subsequent years was variable. The disease, which was not detected east of the river during the winter 2009-2010 epizootic, caused less lamb mortality when it arrived in summer 2010. Since 2013, pneumonia has caused almost complete recruitment failure on each side of the river in at least 1 of the years monitored (Fig. 7).

Although all animals tested possessed the same strain of *M. ovipneumoniae* and all sub-groups have shown at least some evidence of poor lamb recruitment, sub-groups have displayed heterogeneity in the timing and severity of lamb recruitment failures (Fig. 7). This suggests variation in transmission to lambs, which could either reflect movement of animals among sub-herds, or temporal heterogeneity in immune response (Plowright et al. 2013). Co-infections with *Pasteurellaceae* (Table 2) or other aerobic or anaerobic bacteria could also play a role in disease severity. It would require additional concurrent sampling and monitoring to clarify

the reasons for the heterogeneity in long-term response we've noted among these sub-herds. The Yakima Canyon case-study is also noteworthy in exemplifying the difficulty biologists and veterinarians can face in detecting the presence of *M. ovipneumoniae* in adult animals and shows that serology is a more sensitive test than PCR for classifying the *M. ovipneumoniae* status of a population. In our 2013 sampling, we detected *M. ovipneumoniae* in nasal mucosae in none of 31 animals (and only confirmed the bacteria's presence in 1 of the 31 animals by testing for it in nasal washes from the 2 animals with levels considered indeterminate). In combination with our finding only 2 of 17 positive results during 2015 sampling, our best estimate is that prevalence of *M. ovipneumoniae* shedding in adults during the 2013-2015 period of poor lamb recruitment was 6%. At the same time, all 7 lambs collected lethally or found dead during May-September were *M. ovipneumoniae*-positive on PCR.

Asotin herd

As of 2016, it appears that the Asotin herd, although still small, has emerged from the disease event of early 2012, and is free of both *M. ovipneumoniae* and respiratory pneumonia (Table 4). Lamb recruitment 3 and 4 years post-outbreak has returned to pre-outbreak levels, and extensive capture and testing during 2012-2015 indicated very little if any *M. ovipneumoniae* circulating in the population. It is possible that our removal of a few positive animals, after the outbreak when prevalence of carriers was low, purged the population of carriers. The possibility that, with a small-sized herd, carriers died out without our intervention also cannot be ruled out. So far the population has experienced 4 consecutive healthy years after the outbreak. Since 1995, no other population in Hells Canyon has had more than 3 years of good lamb recruitment before recurrence of pneumonia (Manlove et al 2016). However, Asotin is part of a metapopulation of interconnected *M. ovipneumoniae* positive herds. A few more years of monitoring will be useful to assess longer term health status.

Tieton herd

Although it is impossible to know what would have occurred had we not euthanized the remaining Tieton herd individuals, available evidence suggests that neither pathogen nor disease had spread to the adjacent Cleman Mountain herd as of 2016 (Table 4). Based on proximity, and documentation of movement of sheep between the two herd ranges – as well as our experience in the Yakima River Canyon -- we believe that *M. ovipneumoniae* would likely have spread to the Cleman Herd and caused substantial mortality, had we not removed the remaining Tieton animals.

Our decision to remove the entire Tieton herd was not made lightly. Further (but not surprisingly), it was difficult to achieve in practice. It required 6 months and considerable effort to remove the remaining 57 sheep and much was learned. By mid-May when we were able to fully mobilize, the surviving individuals had begun retreating to rugged and forested terrain where finding and removing them was more difficult than the lower-elevation and more open habitats seemingly favored during winter.

One reason we contracted with USDA Wildlife Services to implement the removal was that most WDFW staff were equipped for humanely euthanizing animals at short distances. In contrast, Wildlife Services' use of a helicopter-based shot-gun marksman allowed relatively rapid disposition of animals even in the more remote areas. That said, even with this approach sheep became more difficult to remove with time. Wildlife Services helicopter-based shooting removed 24 animals during the first day but none thereafter, and all subsequent removals resulted from ground-based shooting due to a combination of poor weather and wary

behavior in response to helicopter flights. We speculate that bighorns in this herd were particularly sensitive to helicopters because some had been captured 3 of the previous 4 years by helicopter net gunning.

Following the departure of USDA Wildlife Services, we used standard trail cameras baited with salt to assess how many sheep were left in the area and location. We found that cameras helped us understand how many animals remained, but, because we were able to check them on foot only every 7-10 days, were of little help to shooters attempting to find and remove them. Cameras that send real-time images to computers or cell phone would have been useful.

We found the use of general hunters of limited utility in removing the last few remaining animals. Permitted tribal hunters realized quickly that remaining sheep were scarce, wary, and few, if any, were large rams. We believe this would have been the case with any non-selective process (i.e., had we permitted non-tribal hunters in addition). Instead, our success with non-WDFW staff in removing the few remaining animals depended on soliciting and selecting hunters with the specific motivation, equipment, and skills. The last ewe had become extremely wary of hunters and would not allow anyone within range of most rifles. We were fortunate in having an individual capable of consistent, lethal shots at >800 meters.

Management implications

Review of data collected during the outbreak and post-outbreak monitoring have provided some insights into how bacterial bronchopneumonia may be maintained and spread among bighorns during both invasion and persistent phases of the disease. Our data are far from capable of providing a thorough answer, but they can be used to support or explore various hypotheses. In both the Asotin and Yakima Canyon case studies, we documented widespread intra-herd transmission of *M. ovipneumoniae* during the acute invasion phase. Thus far, patterns observed in the persistence phase in both case studies are consistent with, although insufficient to confirm, the super-shedder hypothesis (Lloyd-Smith et al 2005, Fortin and Cassirer 2015). Data from Yakima Canyon suggest that infection status of individuals can change over time (several years). In both populations, we noted a return to healthy lamb recruitment coincident with documenting a low prevalence of *M. ovipneumoniae* carriers in the population. In the smaller Asotin herd it appears that the only carriers were removed and/or died or recovered naturally and the population is healthy. In the larger Yakima Canyon herd, which continued to experience poor (if heterogeneous) lamb recruitment due to pneumonia-induced mortality at least 6 years post-outbreak, carriers remain at low prevalence.

With the Yakima Canyon herd still large, we have time to attempt some experimentation, but the large size of the herd makes some potential options particularly difficult. Although removing any animal identified as a super-shedder would be possible, it would be prohibitively difficult to capture and test all of the estimated 173 ewes currently in the population (to say nothing of testing positive animals repeatedly). However, the sampling conducted during 2013 and 2015 suggests that the proportion carrying *M. ovipneumoniae* in their respiratory tracts, and presumably being responsible for disease persistence, is quite low. We do not know what percentage of 1 year-old animals are shedding or are in contact with lamb groups in Yakima Canyon (although see Manlove et al. 2017 for evidence that, in Hells Canyon, contact from infected yearlings rarely resulted in disease). To minimize the logistic challenges of large herd size, we could elect to perform experiments only on 1 or 2 sub-herds or on one side of the river. A complication is

that there may be sufficiently frequent movement among sub-herds that even should all the super-shedders within any focal sub-herd be identified and removed, the disease could be reintroduced from a separate, unstudied sub-herd. However, the spatial variability we observed in recruitment suggests that separation of sub-herds during lamb-rearing may restrict or delay pathogen transmission by chronic carriers.

As one of the focal herds in the inter-state Hells Canyon Initiative, research will continue on the Asotin herd, including continued health and demographic monitoring. Animals testing positive for *M. ovipneumoniae* will be removed (non-lethally if a captive facility can use them for research purposes). If no carriers are found and the lamb recruitment continues to show no evidence of pneumonia-related mortality, this will represent only the second population in Hells Canyon in over 3 decades that has cleared disease following a pneumonia outbreak (Coggins 1992, Cassirer et al, 2013, Manlove et al. 2016). Further testing of the super-shedder hypothesis is planned or ongoing in other populations and will help determine whether fadeout can be assisted by management actions or whether this was a natural stochastic purging of the pathogen.

That the Tieton herd exhibited rapid growth and attained a relatively high density (6.2 sheep per km^2) within a few years' time following reintroduction suggests that habitat conditions there were excellent. However, the continued presence of domestic sheep legally grazing on nearby federal allotments poses a risk of future pathogen transmission. In addition to the domestic sheep WDFW documented on core Tieton range just prior to the outbreak, the lessee lethally removed stray domestics in at least 2 other years. It is therefore prudent to await additional assurance that Tieton-herd bighorns would not come into contact with domestic sheep before conducting a reintroduction.

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