

## **Situational Agency Response to Four Bighorn Sheep Die-offs in Western Montana**

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**Abstract:** All-age die-offs occurred in 4 bighorn sheep (*Ovis canadensis*) populations in western Montana during the winter of 2009-2010. Montana Fish, Wildlife and Parks (MFWP) personnel became aware of the first die-off in the East Fork of the Bitterroot (East Fork) population in late November 2009. Subsequent die-offs in the Bonner, Lower Rock Creek and Upper Rock Creek populations became apparent in January 2010. MFWP personnel attempted to actively manage outbreaks by lethally removing (culling) 80 symptomatic bighorns in the East Fork and 99 in Bonner to prevent the spread of the disease to healthy herd segments and neighboring populations. We documented 5 additional bighorn carcasses in Bonner and 6 from the East Fork as potential pneumonia mortalities. MFWP personnel allowed the disease to run its course in Lower and Upper Rock Creek, but removed 48 symptomatic sheep from these populations for diagnostic purposes. All animals collected were necropsied and biological samples were obtained to test for pathogens. Comingling of wild bighorns with domestic sheep or goats was reported post hoc in the East Fork and Bonner, and the East Fork domestics were tested for pathogens. *Mycoplasma ovipneumoniae* was commonly detected utilizing Polymerase Chain Reaction techniques on lung tissue from all bighorn populations, and *Pasteurella multocida* was commonly isolated from the East Fork, Lower Rock Creek, and Upper Rock Creek samples, but not from Bonner in 2009-10. Based upon recent tests using cELISA on banked serum collected in 2007, these bacteria were not detected in the Lower Rock Creek and Bonner populations, but *M. ovipneumoniae* was detected in the East Fork samples. Baseline data were not available for the Upper Rock Creek population. In 2009-10, *M. ovipneumoniae* was detected in pharyngeal swabs from 4 of 7 domestic sheep tested in the East Fork. Prescriptively culled bighorn populations declined by 53-68%, while those populations where bighorn removal occurred only for diagnostic testing declined by 43-60% by March-April 2010. Culling was most successful in the East Fork, as indicated by stable bighorn numbers, no further evidence of pneumonia, and sustained ratios of 32 lambs:100 ewes surviving into August 2010. In contrast, in Upper Rock Creek where limited culling only occurred for diagnostic sampling, adults continued dying in spring and summer and no surviving lambs were documented into August. Although each

affected population was separated from the others by unsuitable habitat or a gap in bighorn occupancy, the die-off across populations highlighted their seasonal or occasional connectivity, and demonstrated the disadvantage of a connected metapopulation of bighorns should a highly contagious pathogen be introduced.

**KEY WORDS:** bighorn sheep, culling, die-off, disease, epizootic, Montana, *Ovis canadensis*, pneumonia, populations, response.

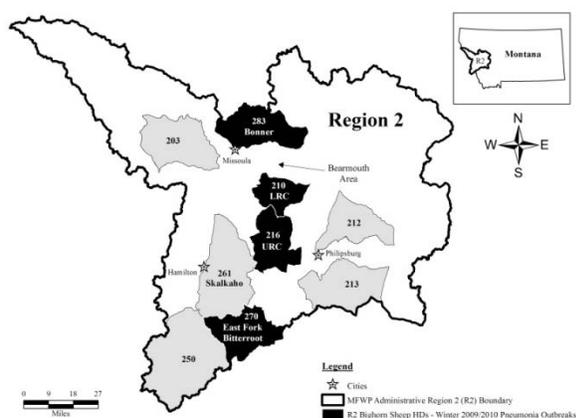
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During the winter of 2009-2010, 5 western states experienced 9 pneumonia epizootics among bighorn sheep (*Ovis canadensis*). Four occurred in western Montana within administrative Region 2 of Montana Fish, Wildlife and Parks (MFWP). Affected Montana populations were from the East Fork of the Bitterroot (East Fork), Bonner, Upper Rock Creek (URC) and Lower Rock Creek (LRC, Figure 1). The last die-off of comparable scale in Montana occurred along the Rocky Mountain Front in 1983 and 1984, though 16 other die-offs have occurred in isolated populations since then (MFWP 2010).

and poor lamb survival and low lamb recruitment may follow the pneumonia event (Onderka and Wishart 1984, Coggins and Mathews 1992, Ryder et al. 1994, Semmens 1996, Aune et al. 1998). *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mycoplasma ovipneumoniae* frequently are isolated from lung tissue of affected bighorn sheep. However, the roles of these bacteria and other factors, such as respiratory viruses, parasites, and stressors such as malnutrition and competition are not clear. Contact with domestic sheep or goats have preceded some bighorn pneumonia outbreaks and, in other cases, contact could not be demonstrated.

Few wildlife management agencies have tested culling techniques to increase survival rates of bighorns during die-off events. Schwantje and Garde (Ministry of Water, Land, and Air Protection, unpublished data) reported that wildlife managers dispatched severely symptomatic sheep during a pneumonia outbreak in the South Okanagan metapopulation of California bighorns (*O. californiana*) in British Columbia. Six of the 8 treated subpopulations appeared to recover quickly, though it was not evident whether the recovery was due to culling or coincidence. During the 2009-10 die-offs, Montana, Washington and Utah implemented culling



**Figure 1.** Map of bighorn sheep hunting districts and populations with pneumonia outbreaks during winter 2009-2010 in Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana.

Pneumonia is a significant mortality factor in bighorn populations throughout the western states. Large-scale die-offs can reduce populations to just a few individuals,

strategies. This paper describes methods and outcomes in Montana.

MFWP personnel implemented 3 response strategies to the die-offs as they unfolded in western Montana in 2009-10, including: selective culling, a combination of selective culling and containment zone culling, and limited culling with removal of only sick animals for diagnostic sampling. The strategy selected depended on the specific circumstances for each population and expression of disease in each locale. As described in the Montana Bighorn Sheep Conservation Strategy (MFWP 2010), every die-off is a unique event, with multiple and dynamic variables for wildlife managers to evaluate when determining a response strategy. These factors include the scope (extent and number of animals affected) of the die-off, stages of the die-off at the time of diagnosis, connectivity of infected bighorns with adjacent herd segments or populations, access to the area where the die-off is occurring, visibility of symptomatic bighorns to the public, time-of-year when the outbreak occurred, and seasonal distribution of the sheep. The protocol outlined in the Strategy allowed flexibility in determining the Agency's course of action, but it also included a recommendation to remove symptomatic animals during early stages of an outbreak in an attempt to reduce the extent of the die-off (MFWP 2010).

In the East Fork and Bonner populations, MFWP personnel culled bighorns to minimize the potential for contact and possible disease transmission between infected and healthy animals. MFWP personnel did not prescriptively cull in the LRC population due to hazardous, snow-covered terrain, or in URC due to the advanced scope of the infection in that population. However, we dispatched limited numbers of bighorns in LRC and URC for

diagnostic evaluation of the pneumonia event.

The 4 case histories provide opportunities to examine 3 working hypotheses: 1) culling infected or exposed bighorns decreases the spread of pneumonia to healthy animals; 2) two or more pneumonia outbreaks were related; and 3) lamb recruitment in subsequent years can be improved by removing symptomatic animals during a pneumonia outbreak.

## STUDY AREAS

### East Fork

The East Fork population was located 5 miles southeast of Darby, Montana, in Hunting District (HD) 270 (Figure 1). More detailed descriptions of the 4 study areas are found in MFWP (2010). In 1972 MFWP reintroduced 19 bighorns to historic bighorn habitat in the Tolan Creek area and 35 into Bunch Gulch, all from the Sun River, Montana. The population responded quickly and MFWP allowed hunting in 1976. In recent years, license numbers ranged from 6 to 8 for either-sex and 10 to 20 for adult ewes.

During spring trend surveys in March 2006 and April 2009, MFWP personnel obtained a record-high count of 246 bighorns and a count of only 187, respectively. Population density prior to the die-off was indexed at ~1.54 bighorns per mi<sup>2</sup> across ~121 mi<sup>2</sup> of occupied habitat. Population objectives were to manage for 200 sheep +/- 20%. Lamb recruitment ranged from 35 to 40 lambs:100 ewes in good years and 18 to 25 lambs:100 ewes in poorer years, with a ratio of 39:100 recorded in 2009.

In 2002, 23 bighorns were translocated to Utah and 14 were moved to the Highland Mountains in Montana. One ewe tested positive for *Brucella ovis* in 2002; however, it was later determined to be a false positive. In 2004, 15 sheep were sent

to Colorado for a challenge study. During the 2002 and 2004 capture operations, contagious ecthyma, or sore mouth, was detected in the East Fork herd by MFWP lab personnel who observed scabs on 1 adult ewe in each of those years.

The latest translocations from the East Fork population occurred in 2007 when 25 bighorns were moved to Utah. All 25 sheep tested negative on serology for *B. ovis*, *Brucella abortus*, Bluetongue, Anaplasmosis, Infectious Bovine Rhinotracheitis, and Bovine Viral Diarrhea Type I and Type II. Seven sheep (28%) had low titers ranging from 1:8-1:16 for Bovine Respiratory Syncytial Virus (BRSV), and 24 (96%) had low titers (1:8-1:32) for Para-Influenza 3 (PI3). *B. trehalosi* was isolated from 18 (72%) of the pharyngeal swabs, and beta-hemolytic *Streptococcus* spp. from 20 (80%) of the swabs. *M. haemolytica* was isolated from 3 (12%) of the swabs. Other organisms that were isolated included *Bacillus* spp., *Enterococcus* spp., *Actinobacillus* spp., *Arcanobacterium pyogenes*, and *Staphylococcus* spp. *M. ovipneumoniae* was not detected from any of the pharyngeal swabs from the East Fork of the Bitterroot in 2007, but Polymerase Chain Reaction (PCR) techniques were not available at that time to detect the presence of *M. ovipneumoniae*. Recent tests using cELISA on banked serum indicated that 19 out of 25 samples (1 result undetermined) were considered seropositive for exposure to *M. ovipneumoniae*. Eight sheep (32%) had low levels of *Nematodirus* spp. Most sheep had coccidia (*Eimeria* spp.), and of those 5 (20%) had high loads. Lungworm larvae (*Protostrongylus* spp.) were found in 75% of sheep, but all had low burdens.

Two large domestic sheep operations were located about 15 miles north of bighorn range. Additionally, there were numerous hobby producers of domestic sheep and goats in the Bitterroot Valley,

including one within bighorn range in Whiskey Gulch. Comingling of wild sheep with those domestics was reported on 3 separate occasions by the public in August and September 2009. MFWP personnel responded to 1 of the reports but were unable to find the bighorns, and the 2 other reports did not reach MFWP personnel before the bighorn die-off began.

#### **Bonner**

The Bonner population was located northeast of Missoula, Montana, in HD 283 (Figure 1). In 1987, MFWP reintroduced 14 wild sheep to historic bighorn habitat on Woody Mountain from URC, and in 1990 added 30 bighorns from the Sun River in Montana. Bighorns soon became well established in all suitable habitats near the community of Bonner. A subpopulation inhabited portions of the Rattlesnake Wilderness and National Recreation Area, and another occupied the area south of the Blackfoot River between Bonner and LaFrey Creek. In 1996, MFWP implemented its first limited-license hunting season. From 1996 to 2009, license levels varied from 2 to 10 for adult ewes, and 1 to 3 for either-sex.

The population objective was 100 bighorns (+/-10 %) as reflected by a spring survey target of 90-110. Survey results ranged from 35 sheep in 1991 to 128 in 2007, and MFWP counted 94 bighorns in May 2009. Population density prior to the die-off was indexed at ~3.76 bighorns per mi<sup>2</sup> across ~25 mi<sup>2</sup> of occupied habitat. During good years, recruitment ranged from 45 to 55 lambs:100 ewes, but lamb:ewe ratios often fell below 35:100. Low ratios in 2008 (28:100) and 2009 (25:100) may not reflect lamb recruitment across the population. In 2008, a large band of ewes, lambs, and young rams was unclassified, and in 2009 MFWP personnel conducted the survey when ewes were lambing and therefore, less observable than usual.

Ground classifications of yearlings during the summer were in the mid-thirties for both years.

In December 2009, MFWP personnel estimated the population at 160-180 bighorns and had considered translocating sheep from this population before the die-off occurred. Human-bighorn conflicts were especially prevalent in the West Riverside community where  $\geq 98$  bighorns grazed on residential lots. Numerous domestic sheep and goats were present for many years as hobby flocks and commercial operations, but there had been no previously known incidence of pneumonia in the Bonner population. After the die-off was detected in January 2010, the public reported a case of bighorns and domestics comingling in the fall of 2009.

Four translocations of bighorns from Bonner occurred over a 10-year period, including 27 to Utah in 2007. All 27 sheep from the translocation tested negative on serology for *B. ovis*, *B. abortus*, Bluetongue, Infectious Bovine Rhinotracheitis, and Bovine Viral Diarrhea Type I and Type II. Six sheep (22%) had a titer for Anaplasmosis, and 8 (30%) had a titer for BRSV. Most BRSV titers were 1:8, but 1 sheep had a 1:32 titer. All sheep had a titer for PI3, ranging from 1:8 to 1:64 for most sheep, but 2 sheep had titers of 1:128. *B. trehalosi* was isolated from 25 (93%) pharyngeal swabs. *Streptococcus* spp. was isolated from 21 (78%) pharyngeal swabs, and *M. haemolytica* was isolated from 7 (26%) swabs. *Staphylococcus* spp. and *Bacillus* spp. were occasionally isolated. *M. ovipneumoniae* was not detected among the pharyngeal swabs from Bonner in 2007, but PCR techniques were not available to evaluate the samples. However, exposure to *M. ovipneumoniae* was not detected in banked serum tested in 2009 utilizing cELISA. Fourteen of the Bonner sheep (52%) had coccidia (*Eimeria* spp.); most had

low burdens, but 3 had burdens that were considered moderately high. Twelve (44%) of the bighorns had low levels of lungworm (*Protostrongylus* spp.), and 11 (41%) had a low burden of *Nematodirus* spp.

#### **Lower Rock Creek (LRC)**

The LRC population was located about 20 miles southeast of Missoula, in HD 210 (Figure 1). MFWP introduced 25 sheep to historic bighorn habitat in LRC from Wild Horse Island in 1979 and added 28 sheep from Lost Creek (near Anaconda, Montana) in 1987. Either-sex license numbers have ranged from 1 to 10 since 1986, and ewe licenses have ranged from 0 to 30 annually.

The population objective was 200 bighorns (+/- 20%). The population grew to 44 by 1983 and peaked at 268 bighorns observed in 1996. In 2008, MFWP personnel observed 201 bighorns during the annual aerial survey of winter range, which we consider to be the baseline number going into the 2010 die-off. (A low count in 2009, was thought to be an anomaly due to a late survey.) Population density prior to the die-off was indexed at  $\sim 2.87$  bighorns per  $\text{mi}^2$  across  $\sim 70 \text{ mi}^2$  of occupied habitat. Lamb recruitment from 1983 to 2008 averaged 36 lambs:100 ewes and ranged from 19:100 to 65:100. On 4 April 2008 (the best aerial survey flight prior to the die-off), 44 lambs:100 ewes were observed.

LRC bighorns congregated in numbers occasionally exceeding 100 in small pastures and residential lawns in summer and fall. Domestic sheep or goats were not known to occur in HD 210 when such presence could have been connected to the die-off. However, domestic sheep occurred in small flocks on the northern fringe of LRC bighorn range outside of HD 210.

In 2007, 15 apparently healthy bighorn sheep were translocated along the Green River in Utah. All 15 sheep tested negative on serology for *B. ovis*, *B. abortus*,

Bluetongue, Infectious Bovine Rhinotracheitis, Bovine Viral Diarrhea Type I and Type II and exposure to *M. ovipneumoniae* (banked serum tested in 2009). Eight sheep (53%) had titers for Anaplasmosis, and 1 (7%) had a titer (1:8) for BRSV. Nine sheep (60%) had titers for PI3, ranging from 1:8 to 1:32. *B. trehalosi* was isolated from 14 (93%) pharyngeal swabs. *M. haemolytica* and *Streptococcus* spp. were both isolated from 7 (47%) swabs. *Staphylococcus* spp. and *Bacillus* spp. were rarely isolated. *M. ovipneumoniae* was not detected by culture on pharyngeal swabs from LRC in 2007, but the samples were not evaluated using PCR techniques. Exposure to *M. ovipneumoniae* was not evident in banked serum recently tested by cELISA. Four bighorn (27%) from LRC had low burdens of coccidia (*Eimeria* spp.). Six (40%) had low burdens of *Nematodirus* spp., and all had low burdens of lungworm (*Protostrongylus* spp.).

#### **Upper Rock Creek (URC)**

The URC population was located about 10 miles west of Philipsburg, Montana, in HD 216 (Figure 1). It was a native population that had been supplemented in 1975 with 31 sheep from Sun River, Montana. About 200 bighorns were thought to be in URC before a die-off in 1967 (Berwick 1968). Following that event only 15 were observed on winter ranges, and lamb production was very low for years afterward (Butts 1980). By 1981 the population had rebounded to  $\geq$  128 sheep. The modern hunting era in URC began in 1979 when 1 license was filled. Subsequently, either-sex license numbers commonly ranged from 8 to 16. Ewe licenses were initiated in 1980 and a range of 5 to 40 such licenses were available through 2009.

The population objective was 300 bighorns (+/- 20%). A record high of 347 bighorns was counted in 2007 and sustained at 342 in 2008 and 2009. Population density prior to

the die-off was indexed at  $\sim$ 3.84 bighorns per  $\text{mi}^2$  across  $\sim$ 89  $\text{mi}^2$  of occupied habitat. Lamb recruitment in URC averaged 43 lambs:100 ewes and from 1990 to 2009 ranged from 27 to 58. Eight months prior to the die-off, MFWP personnel observed 342 sheep with 32 lambs:100 ewes.

Bighorns were translocated from URC in 1984, 1987, and 1997, totaling 83 bighorns that were removed from this population. A recent herd health baseline was not available. A translocation was planned for 2010, but canceled because of the pneumonia outbreak. Domestic sheep or goats were not known to occur in URC.

#### **Connectedness of Bighorn Populations**

Although MFWP personnel managed these 4 populations individually, the pneumonia event refocused our attention on known and suspected connections among these and adjacent satellite populations, known as Bearmouth and Skalkaho (HD 261, Figure 1). MFWP personnel first documented bighorns at Bearmouth and Skalkaho 1 year after translocations in LRC (1979) and the East Fork (1972), respectively. DeCesare (2002) reported seasonal movements of radio-collared bighorns (1 ewe, 2 rams) across I-90 between and within the LRC and Bearmouth populations. Unmarked bighorns also were observed in recent years on Bonner Mountain between Bearmouth and Bonner. Therefore, movement of bighorns between the Bonner and LRC populations is likely.

Movement of bighorns between the LRC and URC populations has not been documented; however, occupied ranges of the 2 populations practically adjoin. In the Bitterroot Valley, a connection between the East Fork and the other populations seems comparatively unlikely, except for the Skalkaho satellite population. The first detection of bighorns in the Skalkaho area coincided with the translocation of bighorns to the East Fork, and DeCesare (2002)

documented potential mixing of a radioed Skalkaho ram with East Fork bighorns in the Whiskey Gulch area where the collared ram migrated for a summer.

## METHODS

### Response Strategies

MFWP employed 3 response strategies to the 4 bighorn sheep die-offs: 1) selective culling—culling of symptomatic sheep, 2) containment zone (CZ) and selective culling—culling of all bighorns in a delineated area (CZ) combined with culling of symptomatic sheep outside the CZ, and 3) limited culling—limited lethal removal of symptomatic animals only for the purpose of diagnostic sampling. The strategy selected depended on the specific circumstances for each population and manifestation of the disease.

Each of the responses involved an initial collection of biological samples to identify pathogens, and MFWP personnel implementing selective culling only in the East Fork and selective and CZ culling in the Bonner population. Personnel dispatched bighorns with firearms and, in rare cases, via chemical immobilization (Bonner population only) by darting sheep from the ground using 570 mg of Telazol® reconstituted with 1.7 ml Xylazine (100 mg/mL) per animal. Personnel then euthanized the animal with Euthasol® at a dose of 1 mL per 4.6 kg of body weight. MFWP personnel initially applied immobilization techniques to bighorns in densely, human-populated areas of Bonner because of concerns of discharging high-powered rifles in the wildland-urban interface. However, the technique was discontinued because of potential complications from long induction times, overall efficiency, and human safety concerns associated with darted sheep evading capture and inadvertently injuring a

member of the public or colliding with nearby traffic.

Culling strategies provided a unique opportunity to collect fresh tissue and blood samples during an ongoing pneumonia event. Sample collection occurred in three forms: 1) carcasses were transported to the Wildlife Laboratory (Lab) in Bozeman for necropsy, 2) a mobile lab was established at the culling site and Lab personnel performed thorough necropsies and collected samples, and 3) samples were collected in the field by biologists, Lab personnel and volunteers. Necropsies involved collection of pharyngeal swabs, blood, lung, liver, feces, and lymph nodes, as well as examination for tapeworm and any abnormalities. Pharyngeal swabs were collected using sterile polyester fiber tipped plastic applicator swabs. When the mobile lab was unavailable, biologists and other field personnel conducted tissue collections, which accounted for the majority of those in the Bonner outbreak, but was also used extensively in the other 3 outbreaks. Field personnel were supplied with individual necropsy kits to collect lung, fecal and liver samples, as well as blood and lymph nodes if time permitted. Crews also recorded the date of collection, location, sex and estimated age (in Bonner, ewes older than 4 were classified only as 4+). Each of the necropsy kits was uniquely numbered to maintain sample identification.

Serologic testing was conducted by the Montana Department of Livestock Diagnostic Laboratory in Bozeman, Montana. Pharyngeal swabs were transported in port-a-cul media to Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington for aerobic and *Mycoplasma* culture. Lung samples were collected and frozen until shipping to WADDL for culture and *M. ovipneumoniae* testing using PCR techniques. Banked serum from sheep

captured in 2007 was submitted to WADDL to test for *M. ovipneumoniae* exposure using a recently developed cELISA. Liver samples were frozen, and a subsample was submitted to South Dakota State University Laboratory for determination of selenium levels. Fecal samples were collected for both lungworm and gastrointestinal parasite evaluation. Analyses of these samples were conducted by Veterinary Parasitology Services, Bozeman, Montana.

*Selective culling (East Fork)*—On 15 November 2009, hunters reported an injured ram near U.S. Highway 93 four miles north of Sula, Montana. MFWP personnel dispatched the ram and assumed it was injured from a vehicle collision. On 22 November 2009 another group of hunters reported 2 dead rams along U.S. Highway 93 five miles north of Sula. MFWP personnel responded to the scene and collected the carcasses, but these mortalities did not appear to be the result of a vehicle collision. That same day wildlife officials transported the bighorns to the MFWP Wildlife Lab, and the following day Lab officials confirmed the presence of pneumonia.

MFWP staff immediately began to implement the disease outbreak protocol outlined in the Montana Sheep Conservation Strategy (MFWP 2010). We first defined the extent of the outbreak within the East Fork herd and established a geographic area where it appeared that infected sheep occurred. Next, MFWP officials began extensive coordination and communication among field, regional and state wildlife bureau personnel. MFWP personnel developed a field and media response plan and distributed information to local and statewide media outlets. A critical element in communicating with the media sources was to establish a point person to respond to all information requests. As MFWP personnel began to formulate a culling

response, we brought local groups of sportsmen and other natural resource agencies into the discussion. This was an important component in building a consensus for a field response to the outbreak and to generate interest in gathering volunteers to assist MFWP staff in field activities.

The 2 most critical components of the culling response were removing all sheep showing clinical symptoms of infection, and obtaining quality tissue and blood samples from those culled sheep. MFWP personnel selectively culled bighorns by using 2 to 3 person teams assigned to specific geographic areas. Teams used various modes of transportation depending on the terrain and access, including stock, motorized, and other non-motorized transportation. Once a team observed a sheep, they determined if it was symptomatic and warranted culling. Clinical symptoms included coughing, shaking head, ears drooping, nasal discharge, stilted gait while walking (goose stepping), walking and suddenly bedding down, bedding down facing a cliff, being solitary, reluctance or inability to move uphill, and grazing an area without any significant movement for an extended period. All teams dispatched sheep using head or neck shots at ranges within 100-yards, unless specific conditions did not allow for such shots; then chest shots at longer distances were permitted. Crews packed out heads of rams that were 2.5 years or older. Carcasses were initially delivered to a mobile lab at the culling site, but sampling transitioned to field collections by biologists, volunteers and lab personnel as the operation progressed.

Sheep exhibiting clinical signs of infection became increasingly difficult to find by mid-December 2009. As a result, MFWP used a helicopter to ferry collection teams to more remote areas that held

bighorns and to push sheep uphill as a way of monitoring behavior and increasing the likelihood of discovering sheep in respiratory distress. Several sheep were culled using this method, and during the flights we also observed the carcasses of 6 sheep that were potential pneumonia mortalities. In addition, teams culled 3 adult ewes that appeared healthy to evaluate the effectiveness of the culling, and also to determine if healthy-looking sheep were devoid of infection. Evidence of pneumonia was not apparent during gross field necropsies and lab results confirmed those field observations.

MFWP personnel conducted bi-weekly aerial surveys from late December 2009 through April 2010 to document population trends during the outbreak. These aerial surveys were bolstered by ground surveys during the same time period.

*Containment zone culling and selective culling (Bonner)*—On 12 January 2010, a local resident in West Riverside reported coughing bighorn sheep in the Bonner herd. MFWP personnel responded within the hour, confirmed there were 5 to 6 potentially sick sheep within a band of 11, dispatched a symptomatic ewe and a male lamb, and transported the carcasses to the MFWP Wildlife Lab. That same evening, the MFWP wildlife veterinarian necropsied the animals and confirmed that the bighorns had pneumonia.

The initial response in West Riverside on 13 January and 14 January 2010 replicated the East Fork selective culling approach. On 14 January 2010, a MFWP wildlife biologist surveyed the Bonner population by helicopter to identify the extent of the outbreak. To prescribe a response strategy specific to the Bonner outbreak, we considered the following factors: 1) the outbreak appeared to be localized, with infected bighorns concentrated just east of Mitter Gulch in

West Riverside; 2) the die-off appeared to be in an early stage as the survey revealed symptomatic sheep only in West Riverside, and our first call from the public reported coughing sheep in this extremely visible population on 12 January 2010; 3) due to landscape connectivity within the population and with adjacent populations, the disease could spread quickly from infected bighorns comingling with healthy herd segments, especially since western Montana was experiencing below-average snowfall; 4) access would be challenging because of steep, brushy terrain in the uplands, and many sheep also were residing on numerous, small, private parcels in West Riverside; 5) the Bonner population was highly visible to the public, and any MFWP management actions would be in full view of the public and media; 6.) human safety concerns associated with discharging high-powered rifles in densely developed areas, and 7) the outbreak was occurring during the winter season when bighorn sheep were concentrated on south facing slopes and at lower elevations.

Culling objectives for the Bonner population were 3-fold: to prevent transmission of pneumonia to healthy population segments within the Bonner herd and the nearby Lower Rock Creek population, to dispatch sheep humanely and minimize exposure of animal suffering to the public, and to provide biological samples for diagnosing the disease and pathogens affecting the population. From these objectives, MFWP implemented a more aggressive approach than was applied in the East Fork, and incorporated a combination of culling strategies—containment zone culling and selective culling.

MFWP personnel dispatched all bighorns (both symptomatic and non-symptomatic) within a pre-defined Containment Zone (CZ), and also selectively culled symptomatic sheep outside the CZ.

MFWP personnel defined a CZ as a polygon delineated across a landscape based upon topographical features, known bighorn movements, and flight data of visibly symptomatic sheep. The CZ included the core area containing infected sheep, an outlying area where infected sheep could have comingled with healthy sheep, and about a 1-mile buffer where animals were primarily healthy, but may become infected by any dispersing sheep from the core area. The CZ was a dynamic boundary, defined by field monitoring and altered as necessary to include areas with infected sheep. Outside the CZ, crews observed sheep behavior, and culled those that were symptomatic and any bighorns comingling with them. Crews then reported their findings back to the area wildlife biologist, and if sick sheep were confirmed outside the CZ, the biologist expanded the CZ boundary line. Conversely, if field personnel found only healthy sheep within a segment of the CZ, we readjusted the CZ boundary line accordingly. The overall purpose of the CZ was to contain the pneumonia outbreak to a small portion of the hunting district.

To ensure the public's safety and to decrease the potential for recreationists to displace and disperse bighorns within the CZ, the Lolo National Forest and The Nature Conservancy implemented an emergency resource closure effective 15 January 2010. The closure restricted public use of the CZ and adjacent lands until intensive culling efforts were completed.

The chronology of culling bighorns in the Bonner population occurred as follows: MFWP personnel intensively removed bighorns within the CZ and monitored and dispatched symptomatic sheep outside the CZ for the first 10-days (12 and 22 January 2010); no personnel were on the ground 23 and 24 January to give the sheep a break from the "hunting" pressure; from 25 January through 28

January crews resumed intensive removal of sheep within the CZ, and continued monitoring bighorns on adjacent lands; from 29 January to 5 February, we decreased the number of crews on the ground and the number of days spent in the field; from 6 February – 19 February 2010, we only removed symptomatic sheep from the Bonner population; and from 20 February 2010 onward, MFWP personnel culled sheep only when the public reported seeing an extremely symptomatic animal. During the initial stage of the operation, MFWP personnel delivered carcasses to a mobile lab at the culling site for necropsy and tissue and serum collection by Lab personnel and biologists. As culling activities progressed, MFWP personnel collected tissue and blood samples in the field and then shipped them overnight to the MFWP Wildlife Lab.

Wildlife personnel conducted random ground surveys during March, April and May 2010 to monitor herd health throughout the district, as well as the redistribution and recolonization of bighorns within and adjacent to the CZ. Data collected included the number of sheep observed, classifications, and locations.

*Limited culling (URC and LRC)*—In early December 2009, a member of the public reported seeing a coughing sheep in the LRC population. MFWP personnel responded on 12 December 2009 and dispatched a ewe, but necropsy results were inconclusive. Other reports followed and on 22 January 2010 MFWP personnel culled a symptomatic ewe and made the first diagnosis of pneumonia in LRC. In the subsequent 10 days, MFWP personnel sampled an additional 9 symptomatic bighorn sheep, all of which were necropsied in the field and showed gross evidence of pneumonia. At that point, we decided to let the disease run its course due to the impracticality and risk associated with culling sheep in extremely steep, snow-

covered terrain, as well as hope that spatial segregation of bands would limit the spread of infection. Nonetheless, diagnostic sampling of symptomatic sheep continued, with a total of 19 bighorns collected through 21 February 2010, and another sick ewe was taken on 8 April 2010. Sampling methods in Rock Creek were conducted by MFWP personnel as described for the East Fork and Bonner except the mobile lab was not onsite at any time.

On 29 January 2010 a symptomatic sheep was observed in URC; 3 bighorn were collected the following day and diagnosed with pneumonia. A concerted effort in URC to collect and diagnose sheep resulted in 25 symptomatic bighorn being sampled on 1 day, all of which were necropsied in the field and diagnosed with pneumonia. An aerial survey completed on 8 February 2010 revealed 45% of 174 sheep observed in URC appeared to be symptomatic based on symptoms of respiratory distress. As a result of the high percentage of symptomatic animals, continuous distribution of the herd, and land-owner opposition to culling, the decision was made to let the disease run its course in URC.

### **Spring Trend Surveys and Lamb Production Monitoring**

Post-outbreak population surveys included MFWP's annual, aerial bighorn sheep trend surveys and spring and summer lamb production ground surveys. The MFWP pilot and area biologists conducted trend surveys during optimal observation conditions in a Bell JetRanger helicopter in early spring prior to bighorns moving from their winter ranges. Annual trend surveys provided total, consistent coverage of standardized survey units that incorporated the highest densities of bighorn distribution. Observability varied among habitat types, especially in the Bonner area where bighorns used dense coniferous forests. MFWP has not developed sightability

models or indexes for the East Fork, Bonner and Rock Creek populations, but trend data provided information sufficient to determine if population objectives were being met (MFWP 2010).

Biologists conducted spring trend counts on 11 March 2010 in the East Fork, on 23 April 2010 in Bonner, on 23 March 2010 in LRC and on 23 March 2010 in URC. For each of the populations, personnel counted and classified sheep by age class and sex, and rams were classified further as Class I (yearling), II ( $\frac{1}{2}$  curl to  $\frac{3}{4}$  curl), III ( $\frac{3}{4}$  curl to full curl) and IV<sup>2</sup> (full curl, Geist 1971). The survey units covered core winter/early spring range of each of the populations, including Mittower Gulch east to Wishard Ridge for Bonner, Medicine Tree south and east to Guide-Rye Road for the East Fork, and the entirety of bighorn winter range in LRC and URC from State Highway 38 north to Interstate 90.

To monitor lamb production and potential recruitment into each of the populations, a student conducted ground surveys on foot and by vehicle from May 2010 through August 2010. The student documented and classified the total number of bighorns observed, group sizes and locations, and categorized rams based on horn development. He visually assessed the health of groups by observing sheep, and at times, applying physical stress to the animals. Observations entailed viewing bighorns for 30 to 60 minutes and documenting sheep behavior. Ground herding was conducted to determine whether bighorns would express symptoms of respiratory distress when exerted, and observations of their response were documented.

### **RESULTS**

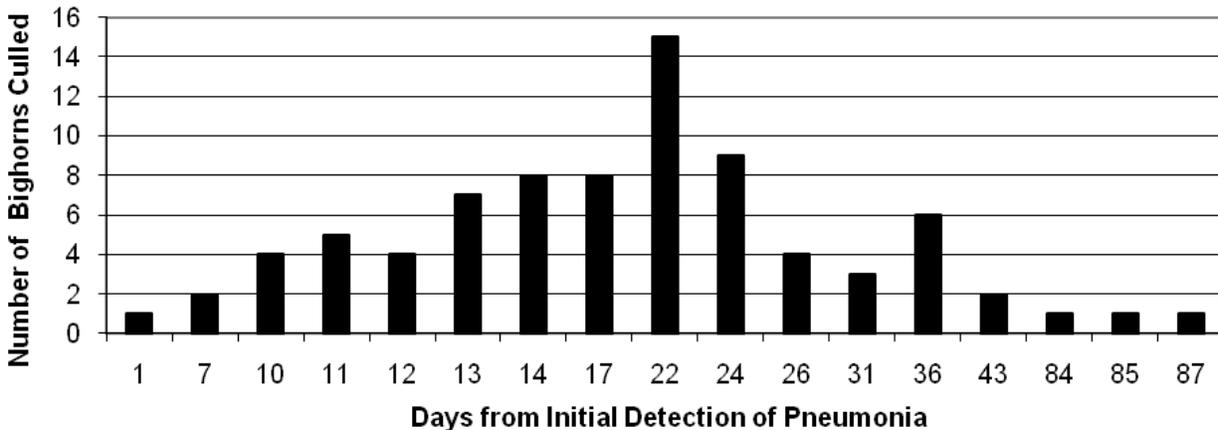
We observed a reduction of 351 bighorns in annual surveys across the 4 affected populations between 2009 and

2010, a decline of 48.5%. If 2009 data are replaced with more representative surveys from 2008 in Bonner and LRC, then a decline of 55.8%, totaling 470 bighorns was documented in 2010. MFWP culled 236 bighorns, or 50.2-67.2% of the decline indicated by the 2010 and 2008-2009 surveys.

**Selective Culling**

Culling of 80 bighorns (46 rams, 22 ewes and 12 lambs) accounted for 81.6% of the observed decline in the East Fork

population. Fifty-eight percent of the culled animals were rams, and 53% of the rams for which age was estimated were younger than 5.5 years old. Adult ewes 4.5 years or older accounted for 36% of all ewes for which age was estimated. MFWP field crews culled the greatest number of sheep on the 22<sup>nd</sup> day (8 December 2009) after the initial discovery of the outbreak, with numbers declining thereafter as personnel observed fewer symptomatic sheep (Figure 2). Ground and bi-weekly aerial observations



**Figure 2.** Number of bighorn sheep culled per day from the initial day of detection of pneumonia in the East Fork of the Bitterroot, Montana, winter 2009-2010.

from late December 2009 through April 2010 indicated no major mortality of sheep after culling efforts ceased in early February. Compared with 2009 spring trend surveys, the 2010 East Fork bighorn counts declined by 53%. Ground surveys indicated stable numbers in the East Fork population in the months after the aerial surveys were completed and MFWP personnel did not observe any bighorns with symptoms of pneumonia through August 2010 (except 1 questionable lamb in early August). Ground surveys post-culling documented lamb:100 ewe ratios of 35 (12:34) on 25 May, 62 (21:34) on 15 June, 30 (15:50) on 14 July and 32 (12:37) on 7-8 August 2010.

Most sheep necropsied early in the outbreak in East Fork were in good to very good body condition. Typical lesions were

severe lung consolidation and fibrinous pleuropneumonia, with the cranioventral aspect of the lung most severely affected.

Most of the East Fork sheep (87.5%) had low titers (1:8 to 1:64) for PI3 and 10% had titers for BRSV (1:8 to 1:32). All sheep were negative on all other serologic tests. Culture results are summarized in Table 1. *M. ovipneumoniae* was detected using PCR techniques in 38/72 (53%) of lung samples from the East Fork. Currently, MFWP Lab personnel are pursuing PCR analysis for detection of *M. haemolytica* in the East Fork lung samples. Preliminary results indicate that *M. haemolytica* may be present in a high proportion of these bighorn sheep lung samples. All but 3 sheep had *Nematodirus* spp. burdens, and 7 of these had high burdens up to 10 times greater than those

**Table 1.** Aerobic culture results of bacteria isolated from samples collected from bighorn sheep in the East Fork of the Bitterroot, Montana, during the winter 2009-2010 pneumonia outbreak.

Type of Sample	Total Samples	<i>Pasteurella multocida</i>	<i>Mannheimia haemolytica</i>	<i>Bibersteinia trehalosi</i>	<i>Pastuerella</i> spp.
Lung	75	41 (55%)	2 (3%)	12 (16%)	0
Swab	37	20 (54%)	5 (13.5%)	26 (70%)	0
Lymph Node	42	26 (62%)	1 (2%)	8 (19%)	1 (2%)
Tonsil	18	7 (39%)	0	15 (83%)	1 (5.5%)

detected in 2007. Sixty-four percent had coccidia (*Eimeria* spp.), and 6 had heavy burdens. Forty percent had lungworm (*Protostrongylus* spp.), nearly all with low burdens. Heavy tapeworm infestation (*Monezia* spp.) was found in several sheep.

All submitted liver samples from the East Fork had liver selenium levels <0.040 ug/g. Normal liver selenium levels have not been established for the bighorn sheep populations involved in these 4 outbreaks. Idaho Department of Fish and Game evaluated liver selenium levels in 8 bighorn populations in Hell's Canyon from 1997-2005. Across populations, liver selenium ranged from 0.03-0.47 ug/g (Cassirer 2005).

A small hobby farm was located near the location of the East Fork outbreak in Sula, Montana. Domestic sheep and goats were among the livestock raised on the property. The owner of the farm allowed MFWP personnel to collect blood samples and pharyngeal swabs from 7 domestic sheep and 2 goats.

One of the 7 domestic sheep had a low titer (1:8) for PI3, but other serologic tests were negative for both domestic sheep and goats. *B. trehalosi* was the most common isolate from pharyngeal swabs of domestic sheep (6/7 or 86% of sheep). *M. haemolytica* was isolated from 3 of the 7 domestic sheep pharyngeal swabs. *Pasteurella* spp. was the only isolate from the pharyngeal swabs of both goats. *M. ovipneumonia* was detected using PCR techniques in 4 of the 7 (57%) pharyngeal

swabs from domestic sheep, but not from swabs of goats.

### Containment Zone Culling and Selective Culling

MFWP personnel dispatched 99 bighorns and collected 5 additional bighorn that appeared to die of natural causes from the Bonner herd. Of the 104 collected, 64 were ewes, 15 were lambs and 25 were rams. Ages ranged from 0.5 to 9.5 years, with rams younger than 5.5 years accounting for 84% of the total rams collected, and adult ewes 4 years or older accounting for 62% of all ewes for which age was estimated. Most bighorn necropsied early in the outbreak were in good to very good body condition.

The spring trend survey reflected a 68% reduction in the number of bighorns observed in the Bonner Survey Unit, from 94 (53 ewes, 12 lambs, 25 rams) to 30 (15 ewes, 8 lambs, 7 rams). In 2009 there were 47 rams:100 ewes and 23 lambs:100 ewes, but survey results were too low in 2010 to calculate the ratios. Sixty percent of the rams observed in 2009 were  $\geq \frac{3}{4}$ -curl, compared with only 29% in 2010. Culled bighorn rams  $\geq \frac{3}{4}$ -curl accounted for about 36% of all the rams collected.

Serology results from culled bighorns in Bonner revealed that 49% of the sheep had a titer for Anaplasmosis and 8% for Bluetongue. Forty-nine percent had a low titer (1:8 to 1:32) for PI3, and 10% had low titer (1:8 to 1:16) for BRSV. Culture results are summarized in Table 2. *M. ovipneumoniae* was detected by utilizing

**Table 2.** Aerobic culture results of bacteria isolated from samples collected from bighorn sheep in Bonner, Montana, during the winter 2009-2010 pneumonia outbreak.

Type of Sample	Total Samples	<i>Pasteurella multocida</i>	<i>Mannheimia haemolytica</i>	<i>Bibersteinia trehalosi</i>	<i>Pastuerella</i> spp.
Lung	87	2 (2%)	0	11 (13%)	3 (3%)
Swab	5	0	0	5 (100%)	0
Lymph Node	33	1 (3%)	0	4 (12%)	3 (9%)

PCR techniques in 79/87 (91%) of lung samples. Sixty-nine percent of Bonner sheep had low levels of *Nematodirus* spp., while 22% had low burdens of coccidia (*Eimeria* spp.). Seventy-one percent of bighorn sheep from Bonner had lungworm; however, only 2 sheep had high egg counts. Several sheep were infected with both *Protostrongylus* spp. and *Muellerius capillaris*. Liver selenium for sheep from this area ranged from <0.040-0.12 ug/g.

Ground surveys indicated lower, and still declining, numbers in the Bonner bighorns after the aerial surveys were completed, and through August 2010, MFWP personnel continued to observe symptoms of pneumonia in a portion of the population. A ratio of 50 lambs:100 ewes was obtained on 23 June 2010 from 12 bighorns in Bonner, with no documented lamb production outside the CZ. Survival of 2 lambs was documented in August 2010.

#### Limited Culling

A spring aerial survey of LRC revealed 19 lambs:66 rams:100 ewes with an observed population decline of 43% (201 vs. 114). Diagnostic removal of 19 sheep in LRC (10 ewes, 8 rams, and 1 lamb) accounted for 21.8% of this decline. The average age of bighorns sampled was 4.5 years for both sexes, with the youngest a lamb and the oldest a 6.5-year-old ram. Body condition of bighorns necropsied varied from poor to good. Ground surveys post-culling documented lamb:100 ewe ratios of 29 (4:14) on 11 May, 8 (5:63) on 31 May-2 June, 17 (4:24) on 10 July and 0

(0:29) on 4-6 August 2010. An unverified report from the public of 4 lambs in late August suggested that some lamb survival escaped detection during the previous ground survey.

Sixty-two percent of the sheep sampled from LRC had low titers (1:8 to 1:16) for PI3. Seventy-seven percent had a titer for Anaplasmosis. All other serologic tests were negative. Aerobic culture results are summarized in Table 3. *M. ovipneumoniae* was detected using PCR techniques in 12 of 18 (67%) lung samples, and in 1 of 3 (33%) pharyngeal swabs. Liver selenium for sheep from LRC ranged from 0.0425ug/g-0.132 ug/g, with a mean of 0.080 ug/g. Two (15%) of 13 fecal samples had low burdens of coccidia (*Eimeria* spp.), 9 (69%) had *Nematodirus* spp., and 11 (85%) had lungworm (*Protostrongylus* spp.). Two of the sheep had high lungworm burdens.

During the 2010 aerial survey in URC, the number of observed sheep was 60% less than in 2009, with 136 observed and ratios of 13 lambs:36 rams:100 ewes. Diagnostic collection of 28 bighorns accounted for 13.6% of the total reduction from the 2009 spring aerial survey. Most of the sheep sampled in URC were ewes (19 of 28), but 6 rams and 3 lambs also were taken. Ages ranged from 0.5 to 8.5 years. Consistent with LRC, summer observations of bighorn lambs declined from a high of 32 lambs:100 ewes to zero (86 bighorns observed in August with no lambs). Ground surveys post-culling documented lamb:100 ewe

**Table 3.** Aerobic culture results of bacteria isolated from samples collected from bighorn sheep in Lower Rock Creek, Montana, during the winter 2009-2010 pneumonia outbreak.

Type of Sample	Total Samples	<i>Pasteurella multocida</i>	<i>Bibersteinia trehalosi</i>	<i>Pastuerella</i> spp.
Lung	18	12 (67%)	1 (5.5%)	1 (5.5%)
Swab	3	1 (33.33%)	1 (33.33%)	2 (66.6%)
Lymph Node	4	4 (100%)	1 (25%)	0

ratios of 16 (5:32) on 28 May, 32 (21:65) on 20-24 June, and 0 (0:72) on 6-9 August 2010. A local rancher in bighorn habitat reported observing dead lambs over the summer, and MFWP personnel observed symptomatic sheep during ground surveys.

Eighteen of 22 (82%) of URC sheep had low titers (1:8 to 1:32) for PI3. Eight (36%) had low titers (1:16 to 1:64) for BRSV. All other serologic tests were negative. Aerobic culture results are summarized in Table 4. *M. ovipneumoniae*

was detected using PCR techniques on all lung samples from URC, and in 14 of 15 (93%) pharyngeal swabs. Liver selenium for sheep from URC ranged from 0.044 ug/g-0.093 ug/g with a mean of 0.0613 ug/g. Fourteen (64%) URC sheep had coccidia (*Eimeria* spp.), 4 of those having heavy burdens. Eighteen (82%) had *Nematodirus* spp., 5 of those having high burdens. Fifteen (68%) of the bighorn had low lungworm burdens.

**Table 4.** Aerobic culture results of bacteria isolated from samples collected from bighorn sheep in Upper Rock Creek, Montana, during the winter 2009-2010 pneumonia outbreak.

Type of Sample	Total Samples	<i>Pasteurella multocida</i>	<i>Bibersteinia trehalosi</i>	<i>Pastuerella</i> spp.
Lung	29	27 (93.1%)	0	0
Swab	15	14 (93.3%)	5 (33.3%)	1 (6.6%)
Lymph Node	1	1 (100%)	0	0

## DISCUSSION

The 2009-10 pneumonia outbreak across western Montana was a dynamic situation, which is continuing and further developing. Results, interpretations and conclusions presented herein are preliminary and not completely informed. However, they reflect our understanding at this time, which we offer for critical review and future reference.

Culling was a management prescription MFWP adopted almost immediately upon first detection and confirmation of pneumonia in the East Fork and thereafter. An essential premise during our culling activities was that pneumonia-

stricken bighorn sheep were likely to die from the disease, and in some populations die-offs greater than 90% of the population may occur (MFWP 2010). MFWP benefitted in its decision-making and implementation from having considered and documented the culling strategy shortly in advance of the outbreak, when developing the Montana Bighorn Sheep Conservation Strategy (MFWP 2010). This planning investment was fortuitous and important for sustaining agency commitment and support for the culling effort over the weeks and months when needed.

Local MFWP personnel responded to the first detection of the first outbreak with varying levels of pertinent training and experience. We had the benefit of a wildlife veterinarian on staff and a centralized wildlife research lab, which delivered skills, materials, and expertise to the field within hours of contact. The first diagnoses of pneumonia were made by the wildlife veterinarian personally after sheep carcasses were collected during the day and delivered and necropsied in the lab overnight. These staff and facilities were essential in providing information to guide decisions and to implement field operations until sufficient experience and expertise had been transferred to field staff and managers. We may not have been able to promptly and effectively implement these unscheduled operations without this centralized service, which provided health surveillance and biological sampling skills practiced statewide on a daily basis.

Selective culling sick bighorns appeared to prevent healthy bighorns from contracting pneumonia at multiple geographic scales. Culling was a race to find and intercept each infected bighorn to limit disease transmission to healthy bighorns, with the probability of contact and disease transmission multiplying with increasing numbers of sick animals on the landscape that were possibly interacting with healthy bighorns. In view of that, culling of infected animals may have helped prevent infected bighorns in the East Fork and Bonner populations from coming into contact with neighboring healthy populations in HDs 203, 250 and 261 (Figure 1). Within the infected populations, culling should have reduced the probability that groups of healthy bighorns would be exposed to infected groups by reducing the length of time that symptomatic individuals mingled with non-symptomatic individuals. Within groups, intensive culling, especially

in the East Fork, seemed effective in protecting healthy individuals from contact with infected animals, but we cannot be certain that non-symptomatic sheep were not carriers of the pathogen(s).

Determining the extent of the outbreak was critical in developing an effective and successful culling response, but was challenging because infected sheep were not always symptomatic. In the East Fork, the majority of the infected population exhibited discernable symptoms, while those in Bonner appeared more moderately symptomatic. *P. multocida* was commonly isolated from samples from East Fork, LRC, and URC, but rarely from Bonner in 2009-10 (Table 1, 2, 3, and 4). (In 2007, *P. multocida* was not cultured from any pharyngeal swabs in the 3 populations sampled.) We speculate that different pathogens evoke different gradients of symptoms within a population, and that culling strategies and their effectiveness vary depending on the pathogens affecting a population.

Serology of the 4 populations revealed that titers for PI3, BRSV, and Anaplasmosis were common; however titers were consistently low, typically ranging from 1:8 to 1:64. It is not uncommon for a proportion of apparently healthy bighorn sheep to have low titers for these pathogens. The fact that the titers were consistently low suggests there has been some level of previous exposure, but is not indicative of current active infection. Titers do not appear to be higher for bighorn with pneumonia in 2009-10 when compared to visually healthy sheep captured in 2007, including the East Fork population.

*M. ovipneumoniae* was commonly detected using PCR techniques on lung tissue from all populations in 2009-10. All mycoplasma cultures of pharyngeal swabs were negative for sheep captured in 2007 in the East Fork, Bonner and LRC herds. Only

the East Fork had any prior evidence of exposure to *M. ovipneumonia* through the testing of banked serum obtained in 2007. Although mycoplasma is extremely difficult to culture, the lack of evidence of exposure on culture or serologic tests suggests that *M. ovipneumoniae* was not present in the Bonner and LRC herds in 2007. Serologic evidence indicates exposure, but not necessarily active infection. The detection of antibodies to *M. ovipneumoniae* in East Fork serum from 2007 suggests that the herd had been exposed to the bacteria; however, no detectable, adverse health effects were observed in the bighorn sheep population prior to December 2009. Although *P. multocida* and *M. ovipneumoniae* were detected during the 2009-10 outbreaks and not cultured from pharyngeal swabs in visually healthy sheep in 2007, we cannot assume that these are the primary agents responsible for the pneumonia outbreaks. Indeed, determining causal pathogens in an epizootic event can be problematic. Recent research has shown that interactions can occur between bacteria and could affect culture results (Dassanayake et al. 2010). These researchers reported that *B. trehalosi* can inhibit growth of *M. haemolytica*, so recovery of *M. haemolytica* from pneumonic lungs by culture could be limited by contact with *B. trehalosi*. This research leads to the question of whether similar interactions occur between other bacterial species.

Our observations that few neonates survived in URC and LRC, and only 2 in Bonner, in the summer following the winter die-off were expected and typical of bighorn die-offs elsewhere (Onderka and Wishart 1984, Coggins and Mathews 1992, Ryder et al. 1994, Aune et al. 1998). Some bighorns  $\geq$  1-year-old in these populations outwardly exhibited symptoms of pneumonia into August 2010. We speculate that non-symptomatic survivors of the outbreak may have been exposed and carried contagious

forms of the causative pathogens, but did not develop fatal infection. As Coggins and Mathews (1992) suggested, maternal ewes appeared to transfer colostral immunity to their lambs, but with a loss of passive immunity over time, neonates developed pneumonia and died shortly afterward. We received reports from the public of numerous dead lambs in URC in summer 2010. The widespread mortality of most lambs in limited culled populations suggests that the pathogens associated with the pneumonia outbreak during the winter persisted in those populations into summer.

Neonates in the East Fork did not exhibit symptoms of pneumonia and they survived into summer at normal rates, which suggests that they did not come into contact with the pathogens associated with the winter pneumonia events. We suggest that the surviving bighorns in the East Fork either did not carry the pathogens that initiated the pneumonia outbreak during the winter or conditions were not conducive for the outbreak to occur, and that culling activities interrupted transmission to these individuals. Although additional testing and monitoring would be required to confirm this, the differing lamb survival rates between the East Fork and Rock Creek populations suggests that the culling effort effectively limited transmission and persistence of pneumonia-causing organisms in that population. We hypothesize that subsequent, annual lamb recruitment in the East Fork population will surpass those of limited cull populations. Unless survival rates should change over the course of the fall, and barring a new introduction of pathogens, we expect the East Fork population to perform as any other healthy bighorn population.

We are hopeful that the benefits of culling in the East Fork will outpace and exceed any disadvantage of having killed symptomatic bighorns that might have

survived pneumonia. Bighorn losses were broadly similar across the selective cull, containment zone cull and limited cull populations by March-April 2010. However, adult bighorns in limited cull populations continued dying into August 2010 and produced few surviving lambs, while adults in the East Fork (selective cull) appeared to maintain normal survival rates and normal ratios of surviving lambs. Therefore, under comparable survey conditions we expect annual survey data to show a further measurable decline in March-April 2011 for limited cull populations and a slight increase in the East Fork. To the extent that adult survivorship in subsequent years was reduced by exposure to pneumonia in 2009-10, or that reduced lamb survival persists for 1 or more additional years, we expect population performance in limited cull populations to fall further behind that of the East Fork until the pathogens responsible for lamb mortalities are eliminated from the populations. MFWP personnel will continue to monitor the 4 affected bighorn populations to test these hypotheses.

Aggressive culling in Bonner did not appear to improve survivability of healthy bighorns within the population. Early detection of a pneumonia outbreak is critical to the potential success of culling, and the Bonner outbreak had achieved a greater critical mass of symptomatic bighorns than what MFWP personnel initially identified. Consequently, the CZ was not effective in isolating the outbreak to the West Riverside area. As a result, a higher proportion of the population was culled in Bonner than in the East Fork, and healthy survivors remained at risk of continued contact with symptomatic individuals into the summer. Also, only 2 lambs were observed in Bonner in August 2010.

Nonetheless, culling in Bonner was successful by measures that reflected unique

circumstances. Culling allowed MFWP to manage the rate, location and appearance of mortality within and near dense human habitation. We used culling to accelerate the die-off within a defined area of seasonally high bighorn concentrations, with the objectives of removing disease in advance of bighorn immigration and outpacing emigration of infected individuals or groups. Also, accelerating mortality reduced public exposure to the die-off event.

As in Bonner, the pneumonia outbreaks in URC and LRC were already well advanced at the point of first detection. The continuing presence of pneumonia in these populations where limited cull strategies were deployed may be a source of infection for surrounding populations in the future.

Public awareness of pneumonia symptoms in bighorn sheep and the value in reporting them was low when the outbreaks first occurred. Early news reports prompted phone calls from the public about observations made weeks or months earlier, which might have resulted in earlier detections, and even prevention of some outbreaks, had we been notified promptly. Continuing media coverage helped, but fell short of prompting some local residents in bighorn habitat to recognize the importance of reporting their observations. In August 2010, we sent a letter to all bighorn hunting-license holders in western Montana, asking them to watch for and report symptoms immediately to MFWP. Currently, we are seeking other avenues for effectively targeting public awareness and response.

We did not measure public opinion. However, our field operations were not hindered by public opposition and were aided by public support. Numerous private landowners in all 4 study areas allowed MFWP personnel access on or across their properties to dispatch and sample bighorns. One landowner in bighorn habitat

voluntarily sold his domestic sheep and goats. A local sportsmen's group publically honored an MFWP biologist with a prestigious award for his work in pioneering the culling effort to save healthy bighorns. Organized groups and members of the general public contributed field assistance. The public respected a closure of public and accessible private land to public recreation within and near the Bonner CZ during culling. Coverage in local newspapers and television was prominent, and we noted letters to editors and internet responses that supported and opposed MFWP's actions. We provided updates and answered questions at regularly scheduled meetings of community groups and others. We do not discount the disappointment, or in few cases, outrage that we had encountered, but generally we worked in an environment of informed public consent, if not support.

We have not demonstrated or dismissed a cause-and-effect relationship between domestic sheep and bighorns during the 2009-10 events. Although recent tests revealed that East Fork bighorns tested seropositive for exposure to *M. ovipneumoniae* in 2007, we cannot conclude they were the source of the bacterium since MFWP personnel did not collect samples in 2007 from the domestic sheep and goats. However, after the 2009-2010 die-off occurred in the East Fork, MFWP personnel received reports of probable comingling occurring between bighorns and domestic sheep and goats over the last several years. In 2009, *M. ovipneumoniae* was commonly detected utilizing PCR techniques on lung tissue from the East Fork population, as well as in pharyngeal swabs from 4 of 7 domestic sheep near the East Fork. Further genetic analysis of the 2009 samples would be required to determine the relatedness of the bacterium identified in both wild and domestic sheep. Tests from samples

collected from all 4 bighorn populations for *M. haemolytica* and leukotoxin are pending.

In retrospect, the 2009-10 die-off in western Montana bighorns was instructive not only in its occurrence, but also in its timing of occurrence. For 3 decades preceding the 2009-10 pneumonia outbreaks, 3 of the 4 affected bighorn populations existed in environments that variably included domestic sheep, goats and other pets and livestock, and occasional comingling of bighorns with domestics was reported or suspected. Yet pneumonia, if it occurred, had minor and undetectable effects on these 4 bighorn populations prior to 2009. To date, we are unaware of any particular or substantive change in bighorns or their environment to explain the timing of the pneumonia outbreaks.

We suggest that a point source (or sources) of disease best explains the original coincidence of disease presence, exposure, contraction, and infection in a susceptible bighorn or bighorns, and that our working hypothesis that 2 or more of the outbreaks were related is correct. Similar microorganisms were identified in diseased East Fork and URC and LRC bighorns, which suggests the possibility of a common source for these 3 outbreaks. *P. multocida* rarely was detected from the Bonner samples, suggesting the possibility of a separate source of infection there (Table 2). Inability to isolate an organism by culture is either due to absence of that organism or to problems with sample collection, handling, storage, or culture technique. Tissue collection and handling protocols were very similar among the culling areas and often carried out by the same individuals. It is our contention that any differences that may have existed in sampling protocols do not account for observed differences in *P. multocida* detected between Bonner and other herds. Coincidentally, MFWP received post-hoc reports of bighorns

comingling with domestic sheep prior to the Bonner and East Fork outbreaks, but not in URC and LRC. Transmission by bighorns dispersing from the East Fork to URC or LRC seemed unlikely without infecting Skalkaho bighorns along the way; however, rutting behavior could explain such unexpected long-distance movements. Following the initial infection, subsequent transmissions from bighorn to bighorn appeared to occur easily across a variety of habitat conditions and involved bighorns of all ages, body conditions, and parasite loads.

Alternatively, evidence also supports a common source for the Bonner, LRC and URC outbreaks. *M. ovipneumoniae* was detected in all 4 populations and was not found in cultures of previous samples from the same populations, although the detection of *M. ovipneumoniae* antibodies in the East Fork cELISA suggests the bacteria was present in this bighorn population as early as 2007. Other bacteria found in sick bighorns were also present in previous samples from their respective populations. If *M. ovipneumoniae* represented a critical commonality across the 4 affected populations, then the absence of *P. multocida* in the Bonner samples may not indicate a source of infection dissimilar from LRC or URC. This reasoning opens the possibility that the Bonner, LRC and URC die-offs may have been initiated from a common source and spread across these readily connected bighorn populations. Under this hypothesis, the East Fork outbreak could have been a separate event and would be consistent with the lack of pneumonia evidenced in the Skalkaho population in 2010. East Fork bighorns have a history of connection with Skalkaho, and likely would have mixed with Skalkaho bighorns had they dispersed to URC (Figure 1).

Population density has been referenced as a possible contributing factor

in predisposing bighorn to pneumonia epizootics (Aune et al. 1998). Three of the 4 affected populations were at or near historic high population levels prior to the outbreaks. However, density dependent stress that would predispose bighorns to infection was not widespread or apparent in lamb recruitment ratios or body condition of culled or sampled bighorns overall. We acknowledge that density may increase emigration rates, which could increase the risk of disease transmission between populations or geographic units within populations. More significant in this study were the extreme within-group densities in affected populations. MFWP personnel observed or received reports of aggregations of over 200 bighorns in URC, over 100 in LRC, and 98 in Bonner in fall 2009. The large group in LRC had gathered around salt blocks placed for horses, and the aggregation in Bonner was within a densely populated residential area. These high within-group densities could greatly increase the rate of disease transmission between bighorns and across populations, and such densities likely played a role in the timing and rapid spread of the 2009-2010 outbreaks. Whether a reduction in population size would result in a reduction in maximum group size is speculative.

## MANAGEMENT IMPLICATIONS

Although there can be benefits of maintaining metapopulations, the die-offs in western Montana demonstrated the disadvantage of a connected metapopulation of bighorns should a highly contagious pathogen be introduced. Although each affected population was separated from the others by unsuitable habitat or a gap in bighorn occupancy, the die-off across populations highlighted their seasonal or occasional connectivity. The die-offs redefined our concept of population connectedness to include the immigration or

emigration of single individuals between populations, whether or not resulting in genetic interchange. Therefore, the occurrence of domestic sheep and goats or any other possible sources of contagious pathogens in bighorn range or along dispersal routes should be treated as a potential source of infection to the metapopulation as a whole. By this definition and with these current awarenesses, at least 3 of the 4 infected populations in western Montana were connected and should have been managed accordingly.

Full implementation of bighorn management by metapopulation in western Montana would differ from past practices. Avoidance of initial bighorn translocations into questionable anthropogenic environments is the best management alternative; however, in areas where bighorns currently reside or disperse, MFWP should use these case histories to open a dialogue with local landowners and communities to effectively identify and manage anthropogenic risk factors to the health and perpetuation of bighorn sheep populations. Because of the extent of human habitation and domestic sheep and goats within and adjacent to the infected populations, MFWP should manage each of the 4 populations within stringent population objectives with the intent to reduce emigration to connected herds, and bighorn translocations should be considered as a possible alternative to connectedness to maintain genetic diversity.

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