

## Investigation of Pneumonia Mortalities in a *Mycoplasma*-positive Desert Bighorn Sheep Population and Detection of a Different Strain of *Mycoplasma ovipneumoniae*

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**ABSTRACT** Discovery of an emaciated and weak ewe on the banks of the Colorado River in northern Arizona was the first indication of a mortality event in the desert bighorn sheep population in the Black Mountains of Arizona. Herein we describe the documentation of an all age die-off due to *Mycoplasma ovipneumoniae* in a population of bighorns that was known to be positive for *M. ovipneumoniae*. We determined that the strain causing the mortality was different from the strain detected in previous surveillance. These results have implications for the management of bighorn sheep populations and disease monitoring.

In August 2015 an emaciated ewe was discovered in the Black Mountains, 5 miles south of Hoover Dam during a boat tour of the Colorado River by Arizona Game and Fish Department (AGFD, Figure. 1). Department personnel euthanized and necropsied the ewe in the field. Gross necropsy findings consisted of bilateral ventral consolidation of lung lobes, thickened bronchi containing white mucoid exudate, fibrinous adhesions between the lungs and the thoracic wall, and multiple white nodules within the lungs. Severe bacterial pneumonia with *M. ovipneumoniae* was diagnosed histologically, and supported by molecular testing of samples. The Black Mountain range is east of the Colorado River and extends from just north of Hoover Dam south to Interstate 40 (Figure 1). During population surveys in late September 2015, AGFD personnel observed 9 sheep carcasses and several sick sheep in the northern portion of the Black Mountains, approximately 7 miles east of the ewe's location. The bighorn sheep population in areas adjacent to the Colorado River was more than 75% below the 5-year aerial survey average.

The survey findings were strongly suggestive of a recent all age die-off, and the decision was made to conduct active disease surveillance in the affected management units (15CN and 15CS) west of US highway 93 and north of State Route 68. In November 2015 we captured and sampled 3 ewes from 3 different groups in the southern portion of the 15CS unit, approximately 30 miles south of the initial case's location. During the capture operations, many of the bighorns were exhibiting signs of respiratory infection, including the 3 sampled ewes. *M. ovipneumoniae* was detected by PCR from nasal swabs and *M. ovipneumoniae*-specific antibodies were detected with enzyme-linked immunosorbent assay (ELISA) in all 3 ewes. Two *Pasteurella* spp. were identified, one each in two of the ewes, and in one of these, the leukotoxin-A gene was detected.

Two weeks later, hunters reported coughing bighorns immediately south of State Route 68 in the adjacent game management unit, (approximately 58 miles of the location of the first ewe). As a result, we conducted additional surveillance in early December by selecting 3 ewes, one from each of 3 groups, for euthanasia. The ewes were in good body condition and did not display outward signs of disease. During necropsy, fresh and formalin fixed samples were collected from the cranial and caudal lung lobes bilaterally, as well as the right middle lung lobe. From the caudal lobes, we collected tissue near the main stem bronchus and the distal margin. The cranial and middle lung lobes of the ewes contained areas of consolidation, with thickened airways filled with mucopurulent exudate. No other abnormalities were detected on the necropsies. All 3 were diagnosed with acute bacterial pneumonia by histology. *M. ovipneumoniae*, *Bibersteinia trehalosi* (moderate to few), and *Fusobacterium necrophorum* (few to very many) were detected with PCR and microbial culture. Additionally, we collected swabs from 12 hunter-harvested bighorn rams from units 15C and 15D. *M. ovipneumoniae* was detected by PCR on 11 of the samples.

The Arizona Department of Game and Fish conducted disease surveillance on captured bighorns in these units during 2012 and in adjacent units annually from 2012 to 2014 during research and translocation projects. At that time, *M. ovipneumoniae* was detected by PCR conducted on nasal swabs in 5-10% of captured bighorns. Because we had detected *M. ovipneumoniae* in this population during these earlier surveillance events, we wanted to determine if the bighorns affected by pneumonia in 2015 were succumbing to the previously detected strain or a new strain, and if a new strain was detected, determine the source of the infection.

We performed strain-typing using partial sequences of four loci (16S, IGS, rpoB, and gyrB) from the *M. ovipneumoniae* in the affected bighorns ( $n = 4$ , Figure 2, Index case and Sampled ewes 1-3) in 2015 and from 3 bighorns from just east of Lake Mead (Figure 2, Grand Wash ewes 1-3) sampled as part of a population health assessment, and compared them to the sequences of *M. ovipneumoniae* detected in prior years and to organisms detected in bighorns captured south of the outbreak in November 2015. The strain identified in samples from 2012 to 2015 matched the strain identified in bighorn sheep sampled in the following southern Nevada mountain ranges in 2013, including the Spring Mountains, El Dorado Mountains, McCullough Range, and River Mountains (Figures 1 and 2). The *M. ovipneumoniae* detected in bighorns with pneumonia in 2015 differed from the previously identified strain and instead matched a strain initially detected in a mortality event at Old Dad Mountain (Providence Mountains, Figure 2, CA 2013 Mohave outbreak) in California in 2013. This strain was later detected during sampling efforts in response to detected disease events in the Spring Mountains (2013), River Mountains (2014), and Eldorado Mountains and McCullough Range (2015) of Nevada (Figure 2, NV2013-4 Mohave outbreak). Additionally, IGS sequences from the 3 euthanized ewes and 5 of 5 hunter-harvested rams matched the IGS sequence of the Mohave outbreak strain.

Prior to the detection of pneumonia in the ewe found on the Colorado River and the identification of mortalities during the subsequent fall surveys, the bighorn sheep population in the Black Mountains of Arizona was considered to be productive despite the presence of *M. ovipneumoniae*. The occurrence of respiratory disease in conjunction with the detection of a different strain of *M. ovipneumoniae* suggests that strains vary in pathogenicity and that exposure to one strain does not induce a broad immunity across strains. This is consistent with mycoplasma infections in domestic chickens and turkeys, as well as house finches (Kang et al. 2002, Hinz et al. 2003, Grodio et al. 2012), and has also been reported in bighorn sheep *M. ovipneumoniae* pneumonia (Cassirer et al. 2017).

The apparent movement of this strain of bacteria from Old Dad Peak to the Black Mountains as indicated by the sequential detections from California east to Nevada and Arizona suggests that the disease was likely introduced by the natural movement of bighorn sheep. Repeated introductions of the identical strain of mycoplasma into multiple populations of bighorns from multiple exposures to domestic sheep and goats are unlikely as mycoplasmas undergo frequent strain differentiation (Besser et al. 2012, Tulman et al. 2012, Spargser et al. 2013, Sulyok 2014). The events documented in this report provide additional clues to the epidemiology of bighorn sheep pneumonia and provide support for routine monitoring of bighorn sheep populations for the occurrence of pathogens with the inclusion of strain-typing and geospatial epidemiological modeling including population interaction parameters.

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**KEYWORDS** Bighorn sheep pneumonia, *Fusobacterium necrophorum*, molecular epidemiology, *Mycoplasma ovipneumoniae*, strain-typing.

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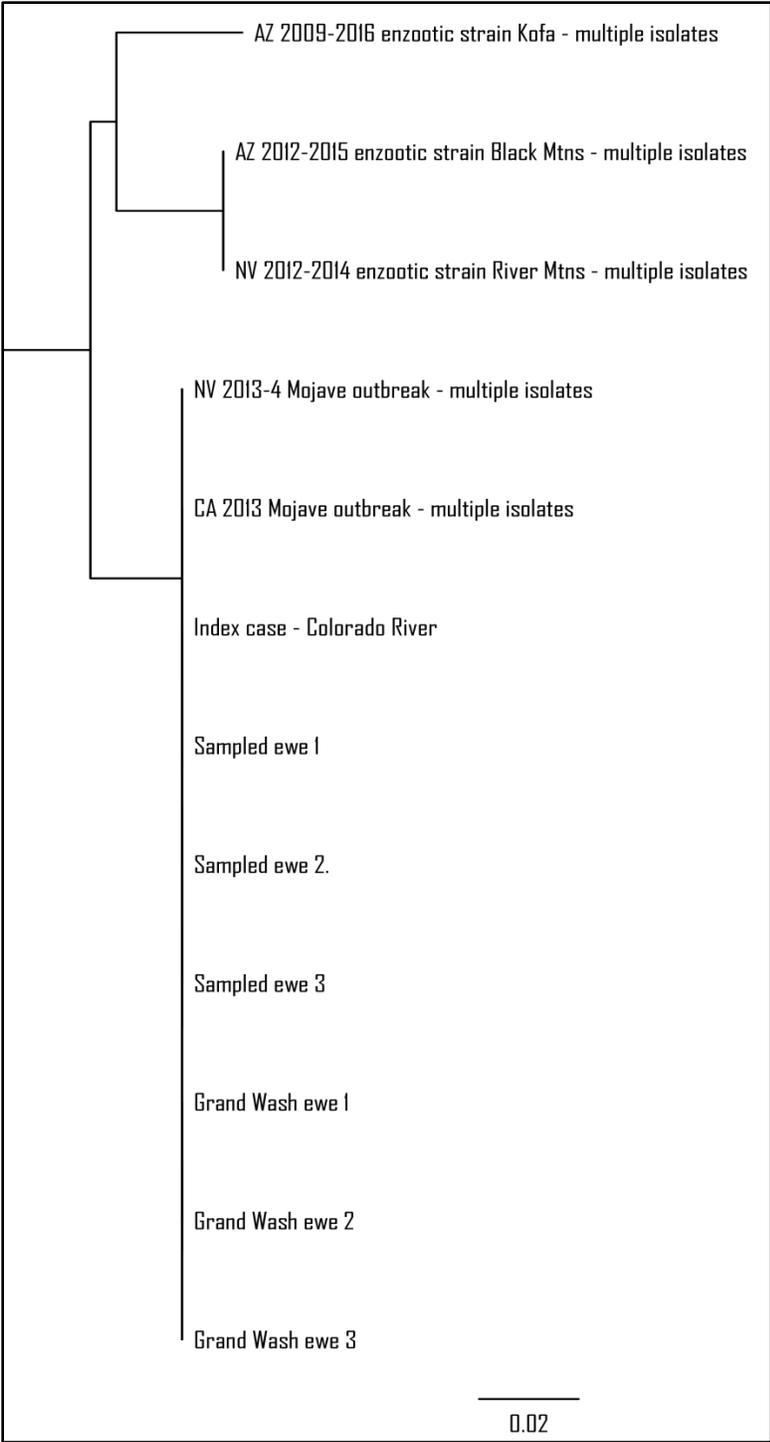


Figure 1. Location of disease event cases in the Black Mountains of Arizona and nearby mountain ranges in California and Nevada.

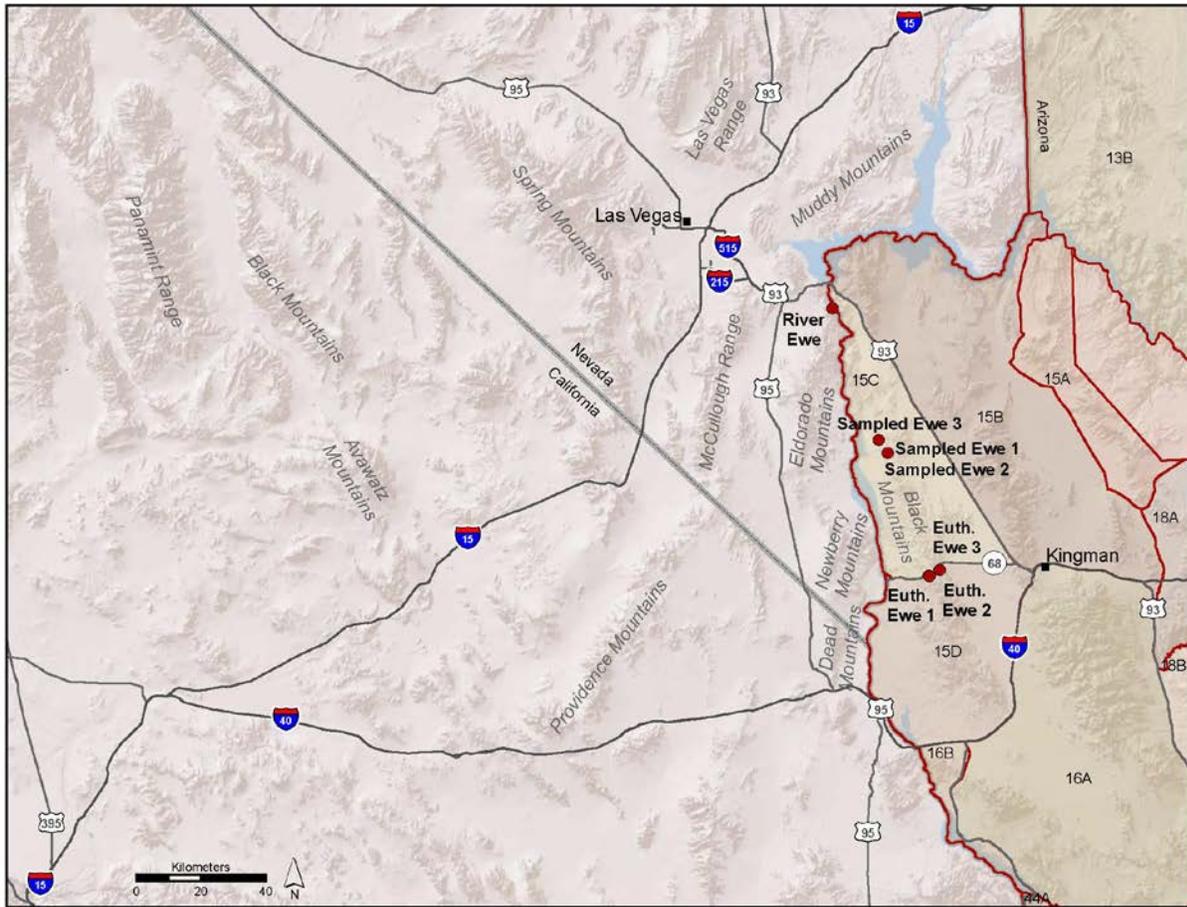


Figure 2. Phylogenetic tree of the *Mycoplasma ovipneumoniae* strains identified in southern California, Nevada, and Arizona prior to the all age die-off and from bighorns sampled during active disease surveillance after the die-off.