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# Previously Unrecognized Exposure of Desert Bighorn Sheep (*Ovis canadensis nelsoni*) to *Mycoplasma ovipneumoniae* in the California Mojave Desert

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ABSTRACT: A 2013 outbreak of respiratory disease in bighorn sheep from California's Mojave Desert metapopulation caused high mortality in at least one population. Subsequent PCR and strain-typing indicate widespread infection of a single strain of *Mycoplasma ovipneumoniae* throughout this region. Serosurvey of archived samples showed that some populations have had antibodies to *M. ovipneumoniae* since at least 1986, although pre-2013 strain-type data are unavailable.

Respiratory disease has a long history of demographic influences on bighorn sheep (*Ovis canadensis*) in North America (Wehausen et al. 2011; Cassirer et al. 2018). *Mycoplasma ovipneumoniae* is important in the polymicrobial complex associated with this disease and has become the focus of surveillance efforts (Besser et al. 2012). In naive populations, outbreaks of respiratory disease typically manifest as all-age mortality events, followed by a period of enzootic, self-limiting disease among surviving adults but continued high mortality of neonates (Cassirer et al. 2013; Plowright et al. 2013). Infected individuals often present with pneumonia, and clinical signs include coughing and nasal discharge (Besser et al. 2017). Contact of susceptible bighorn sheep with domestic sheep (*Ovis aries*) or goats (*Capra aegagrus hircus*) or with infected bighorn sheep is the primary source of transmission (Plowright et al. 2013).

Desert bighorn sheep (*Ovis canadensis nelsoni*) populations in the Mojave Desert of California occupy mountain ranges separated by low-lying desert. Historically, these populations were part of a large multistate metapopulation that has been partially fragmented by Interstate Highways 15 and 40 (Fig. 1; Bleich et al. 1996; Epps et al. 2018). During May–July 2013, approximately 30 bighorn sheep carcasses and more than 20 individuals with signs of respiratory distress were detected around Old Dad Peak and the Kelso Mountains (ODKM), north of Interstate 40 (I-40). Postmortem examinations of two carcasses and one euthanized individual revealed severe bronchopneumonia. *Mycoplasma ovipneumoniae* DNA was detected by PCR in lung tissues and upper respiratory swabs from these cases by the Washington Animal Disease Diagnostic Laboratory (Besser et al. 2012).

In August 2013, *M. ovipneumoniae* DNA was confirmed by PCR in five bighorn sheep lethally collected from the Marble Mountains population south of I-40 that were exhibiting clinical signs of respiratory disease (Fig. 1). Multilocus sequence typing (Cassirer et al. 2018) of *M. ovipneumoniae* from two individuals in ODKM and two from the Marble Mountains confirmed the same strain in both populations. This strain was later detected in association with respiratory disease in populations from Nevada and Arizona, US, where it

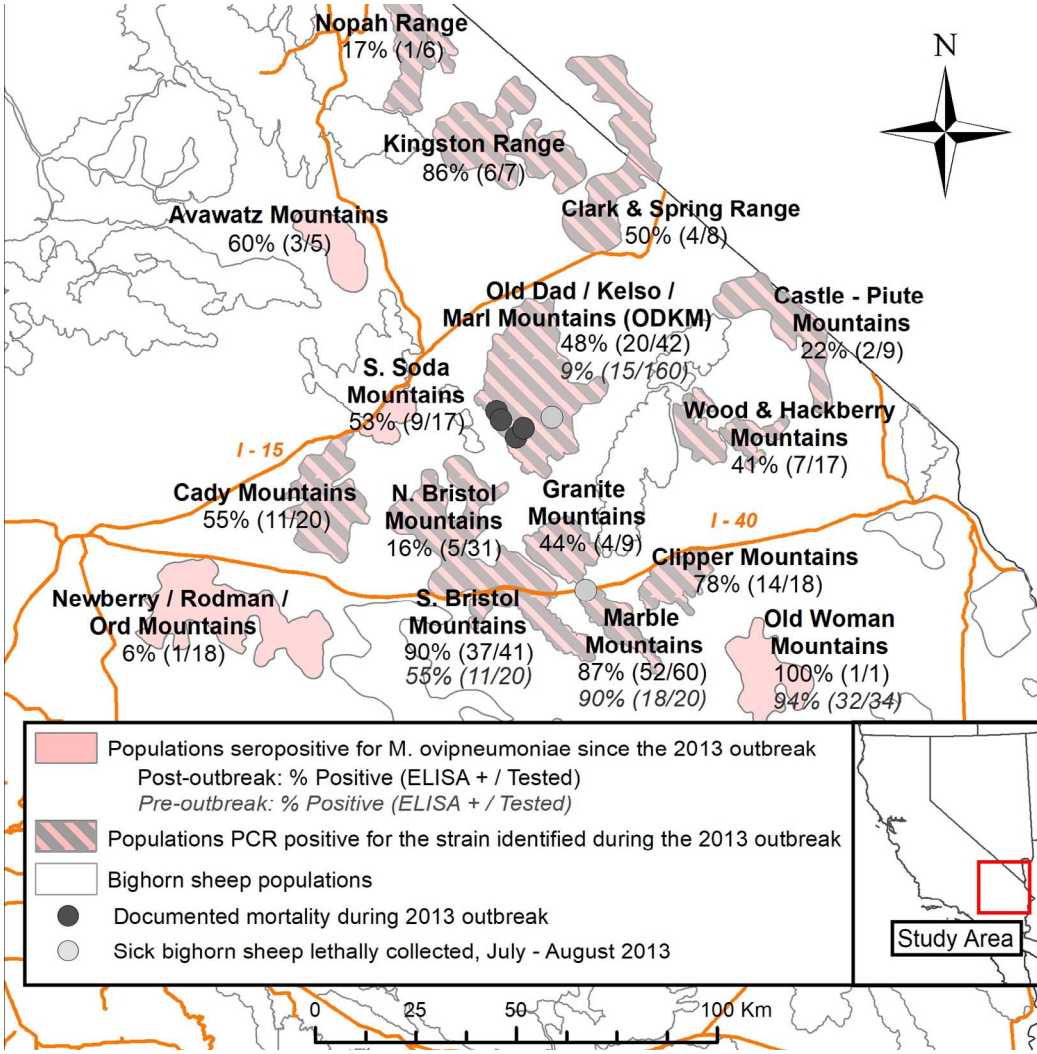


FIGURE 1. Bighorn sheep (*Ovis canadensis nelsoni*) populations in the Mojave Desert of California seropositive for *Mycoplasma ovipneumoniae* by enzyme-linked immunosorbent assay (ELISA) both before and after the 2013 outbreak. Text below population names indicate the percentage of positive results by enzyme-linked immunosorbent assay for *M. ovipneumoniae* (seropositives/no. tested) before 2013 (dark-gray, italicized text), and since the 2013 outbreak (black text). Populations without italicized text were not tested before the outbreak. Gray hatching indicates populations in which individuals that were PCR positive for *M. ovipneumoniae* had identical sequences, at the 16S–23S intergenic spacer region locus, to the strain identified during the 2013 outbreak. Dark-gray circles indicate locations of mortalities during the 2013 outbreak; light gray circles indicate the location of bighorn sheep observed with respiratory symptoms and sacrificed for testing. Major interstate highways 15 and 40 trisect the study area and are denoted in small italicized text (*I-15* and *I-40*).

displaced a less-pathogenic enzootic strain (Justice-Allen et al. 2016).

To ascertain the extent of the outbreak, 91 male and 222 female bighorn sheep were captured in 16 mountain ranges during 2013–15 and 2017–18 (Table 1) via helicopter net gunning and sampled, following California Department of Fish and Wildlife guidelines. Captures were reviewed and approved by the National Park Service Institutional Animal Care and Use Committee (ACUP#PWR_MOJA_Epps.Powers_DesertBighorn_2013.A3, 2013-

2016; _2016.A3, 2016-2019). Blood was collected by jugular venipuncture. Two nasal swabs, one dry and one placed in tryptic soy-broth medium with 15% glycerol (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), were also collected. Swabs and serum were stored on ice and then frozen at –80 C until tested. Serum was screened for antibodies to *M. ovipneumoniae* by enzyme-linked immunosorbent assay, and nasal swabs were tested by PCR for *M. ovipneumoniae* DNA at Washington Animal Disease Diagnostic Laboratory.

Across populations, PCR detections of *M. ovipneumoniae* were greatest during 2013 and decreased over time (Table 1). At ODKM, *M. ovipneumoniae* was detected in nasal swabs from 58% (11/19) of individuals tested in 2013 but has not been detected during subsequent captures ($n$=24). Sequence typing results at the 16S–23S intergenic spacer region locus (Cassirer et al. 2018) of PCR products from *M. ovipneumoniae*–positive populations ($n$=12; Table 1) were consistent with the strain identified at ODKM in 2013.

Spatial patterns of our PCR results and strain type data for populations with repeated samples beginning in 2013 (Table 1; Justice-Allen et al. 2016; Cassirer et al. 2018) suggest widespread infection of a single *M. ovipneumoniae* strain throughout the study area (Fig. 1). This strain was detected on both sides I-40 and I-15 (Cassirer et al. 2018), previously thought to be major movement barriers (Epps et al. 2005) but now recognized to be more porous (Epps et al. 2018).

We detected antibodies to *M. ovipneumoniae* in all populations tested, and most, including ODKM, have maintained high levels of seroprevalence since 2013 (Table 1). We also tested 234 archived serum samples to search for evidence of exposure to *M. ovipneumoniae* in the study area before the outbreak. Antibodies to *M. ovipneumoniae* were detected in three populations south of I-40 in all years tested: Old Woman Mountains (1986–87, 1990, and 2001–02), Marble Mountains (1986, 1990, and 2005), and Southern Bristol Mountains (2002 and 2005; Table 1 and Fig. 1). North of I-40, no antibodies to *M. ovipneumoniae* were detected at ODKM between 1983 and 1988. However, serodetections in 1989 and 1990, but not in 1992 or 2005–06, suggest this population had been exposed, but that infected individuals either left the population, died, or were able to clear their infections. Population-level clearance of *M. ovipneumoniae* has been achieved experimentally by culling chronic shedders (Garwood et al. 2020), but clearance could also occur naturally through emigration, death of chronic shedders, or unintentionally through past management actions, such as translocation. If the same strain of *M. ovipneumoniae* identified in 2013 was responsible for the antibodies detected at ODKM in 1989 and 1990, the absence of antibodies in the intervening years suggests a loss of humoral immunity to that strain. However, bighorn sheep immunity to *M. ovipneumoniae* appears to be strain specific (Justice-Allen et al. 2016; Cassirer et al. 2017), and without knowledge of earlier strain types, it is unclear whether previous exposure to *M. ovipneumoniae* would have conferred any herd immunity to the 2013 strain.

Serologic results from historic samples should be interpreted with caution, given the diversity and variable pathogenicity among strains of *M. ovipneumoniae* (Besser et al. 2017; Kamath et al. 2019). Seropositive results from the Old Woman Mountains and Marble Mountains in 1986 and 1987 coincided with reports of respiratory disease in lambs and poor recruitment, but detections in the Marble Mountains during 1990 and 2005 overlapped periods of lamb recruitment that tracked winter-spring diet quality and when no evidence of disease was observed, despite intensive monitoring (Wehausen 2005). Likewise, seropositive results from ODKM in 1989 and 1990 coincided with a severe drought and subsequent poor lamb recruitment in 1990, which was followed by a rapid return to normal (Wehausen 2005). Without direct detections of *M. ovipneumoniae* from mortality investigations before 2013, it is not possible to definitively associate *M. ovipneumoniae* exposure with historic demographic trends.

A fundamental phenomenon evident in the 2013 outbreak is the great difference among

TABLE 1. Nasal swabs tested for *Mycoplasma ovipneumoniae* DNA by PCR and serum for antibodies to *Mycoplasma ovipneumoniae* by enzyme-linked immunosorbent assay (ELISA) from desert bighorn sheep (*Ovis canadensis nelson*) populations in the Mojave Desert of California, collected from 1983 to 2018, including the number of samples strain-typed at the 16S–23S intergenic spacer region (IGS) locus by year in each population; all IGS sequences reported matched the 2013 outbreak strain. Samples before 2013 had been archived but were screened retrospectively by ELISA only.[a]

| Region and desert bighorn sheep population | Year | IGS strain typing (*n*) | *Mycoplasma ovipneumoniae*, percentage (*n* positive/*n* tested) | |
|---|---|---|---|---|
| | | | PCR | ELISA |
| North of I-15 | | | | |
| Avawatz Mountains | 2018 | — | 0 (0/5) | 60 (3/5) |
| Clark Mountain and Spring Mountain Range | 2017 | 1 | 25 (1/4) | 33 (1/3) |
| | 2018 | 1 | 20 (1/5) | 60 (3/5) |
| Kingston Range and Mesquite Mountains | 2017 | 1 | 14 (1/7) | 86 (6/7) |
| Nopah Range | 2018 | 1 | 17 (1/6) | 17 (1/6) |
| Between I-15 and I-40 | | | | |
| Cady Mountains | 2014 | 3 | 30 (3/10) | 70 (7/10) |
| | 2018 | ND | 20 (2/10) | 40 (4/10) |
| Castle-Piute Mountains | 2018 | 1 | 11 (1/9) | 22 (2/9) |
| Granite Mountains | 2013 | 1 | 40 (2/5) | 40 (2/5) |
| | 2018 | — | 0 (0/4) | 50 (2/4) |
| Northern Bristol Mountains | 2013 | 1 | 17 (1/6) | 33 (2/6) |
| | 2015 | ND | 8 (1/12) | 8 (1/12) |
| | 2017 | — | 0 (0/3) | 33 (1/3) |
| | 2018 | ND | 10 (1/10) | 10 (1/10) |
| Old Dad Peak, Kelso Mountains, and Marl Mountains | 1983 | — | — | 0 (0/15) |
| | 1984 | — | — | 0 (0/15) |
| | 1985 | — | — | 0 (0/15) |
| | 1986 | — | — | 0 (0/10) |
| | 1988 | — | — | 0 (0/19) |
| | 1989 | — | — | 50 (9/18) |
| | 1990 | — | — | 50 (6/12) |
| | 1992 | — | — | 0 (0/30) |
| | 2005 | — | — | 0 (0/9) |
| | 2006 | — | — | 0 (0/17) |
| | 2013 | 9 | 58 (11/19) | 68 (13/19) |
| | 2015 | — | 0 (0/7) | 43 (3/7) |
| | 2017 | — | 0 (0/13) | 17 (2/12) |
| | 2018 | — | 0 (0/4) | 50 (2/4) |
| Southern Soda Mountains | 2013 | — | 0 (0/4) | 50 (2/4) |
| | 2015 | — | 0 (0/6) | 50 (3/6) |
| | 2018 | — | 0 (0/7) | 57 (4/7) |
| Woods and Hackberry Mountains | 2013 | 3 | 60 (3/5) | 50 (3/6) |
| | 2014 | — | 0 (0/1) | 0 (0/1) |
| | 2015 | ND | 25 (2/8) | 25 (2/8) |
| | 2017 | — | 0 (0/2) | 100 (2/2) |
| South of I-40 | | | | |
| Clipper Mountains | 2013 | 3 | 100 (4/4) | 100 (4/4) |
| | 2015 | ND | 17 (2/12) | 75 (9/12) |
| | 2017 | ND | 50 (1/2) | 50 (1/2) |

TABLE 1. Continued.

| Region and desert bighorn sheep population | Year | IGS strain typing (*n*) | *Mycoplasma ovipneumoniae*, percentage (*n* positive/*n* tested) | |
|---|---|---|---|---|
| | | | PCR | ELISA |
| Marble Mountains | 1986 | — | — | 100 (5/5) |
| | 1990 | — | — | 100 (5/5) |
| | 2005 | — | — | 80 (8/10) |
| | 2013 | 4 | 57 (8/14) | 100 (15/15) |
| | 2014 | ND | 11 (1/9) | 78 (7/9) |
| | 2015 | ND | 38 (3/8) | 75 (6/8) |
| | 2017 | ND | 12 (3/26) | 84 (21/25) |
| | 2018 | ND | 33 (1/3) | 100 (3/3) |
| Newberry, Rodman, and Ord Mountains | 2014 | — | 0 (0/4) | 0 (0/4) |
| | 2017 | — | 0 (0/11) | 0 (0/11) |
| | 2018 | — | 0 (0/3) | 33 (1/3) |
| Old Woman Mountains | 1986 | — | — | 100 (5/5) |
| | 1987 | — | — | 100 (5/5) |
| | 1990 | — | — | 80 (4/5) |
| | 2001 | — | — | 92 (11/12) |
| | 2002 | — | — | 100 (7/7) |
| | 2014 | — | 0 (0/1) | 100 (1/1) |
| Southern Bristol Mountains | 2002 | — | — | 50 (5/10) |
| | 2005 | — | — | 60 (6/10) |
| | 2013 | 3 | 100 (13/13) | 92 (12/13) |
| | 2014 | ND | 14 (1/7) | 83 (5/6) |
| | 2015 | ND | 14 (1/7) | 86 (6/7) |
| | 2017 | ND | 30 (3/10) | 90 (9/10) |
| | 2018 | ND | 40 (2/5) | 100 (5/5) |

[a] — = no samples were collected or available; ND = not done, sample not tested.

populations in demographic responses for both adult survival during the outbreak and in lamb recruitment since. Although initial work has shown that ecologic factors, such as forage quality, have a role (Dekelaita et al. 2020), a better understanding of these differences will require simultaneous, long-term data on multiple ecologic, genetic, and disease variables across populations before, during, and after disease outbreaks.

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