Prevalence of *Mycoplasma ovipneumoniae* in desert bighorn sheep in Arizona

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Abstract To assess the potential for an epizootic of pneumonia to result from either natural immigration or translocation, we compared the seroprevalence to *Mycoplasma ovipneumoniae* in several populations of desert bighorn sheep in Arizona. We collected blood samples and nasal or oropharyngeal swabs from 124 desert bighorn sheep (*Ovis canadensis nelsoni*) from 6 populations in Arizona in 2009 and 2010. *M. ovipneumoniae* organisms were detected by PCR in 22%, whereas antibodies to *M. ovipneumoniae* were detected in 47% of tested bighorn sheep. Mycoplasma antibodies were not found in 2 of 6 populations, indicating some bighorn sheep populations in Arizona are naïve to this bacterium. In contrast, others had seroprevalence rates up to 80%. We were able to compare seroprevalence rates and titers over time in 9 individuals (7 individuals included in the 124 bighorn sheep sampled in 2009 and 2010, and 2 individuals originally captured in 2006). Antibody titers persisted for 12 months in individuals from the Kofa National Wildlife Refuge (n = 7) while antibody titers appeared to decline in the Kanab Creek population (n = 2). *M. ovipneumoniae* is present or has been present in several, but not all, populations of bighorn sheep in Arizona. The results demonstrate the importance of routine health testing for future translocation efforts to reduce disease risk for naive populations.

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Infectious diseases, especially pneumonia, influence bighorn sheep (*Ovis canadensis*) populations in the USA. and Canada (Rudolph et al. 2007, Besser et al. 2008, Weiser et al. 2009, Cassaigne et al. 2010). In 2010, epizootics of pneumonia in 9 herds in 5 states killed about 900 bighorn sheep, about 1% of the total number of bighorn sheep in the western United States and Canada (Wild Sheep Working Group,

Western Association of Fish and Wildlife Agencies [WSWG-WAFWA] 2010, Besser et al. 2012*b*). Mortality rates ranged from 5 to 95% (WSWG-WAFWA 2010, Besser et al. 2012*b*). Several organisms have been associated with outbreaks of pneumonia in bighorn sheep, and it is likely that multiple agents are involved in many outbreaks (Dassanayake et al. 2010, Wolfe et al. 2010). One likely causative agent is *Mycoplasma ovipneumoniae* (Rudolph et al. 2007, Besser et al. 2008, Dassanayake et al. 2010, Besser et al. 2012a). In 2009, the Wildlife Health Committee (WHC) of WAFWA introduced a set of guidelines for assessing and monitoring the health status of wild sheep populations as part of translocation management and for general population monitoring (WAFWA 2009). From June 2009–November 2010, the Arizona Game and Fish Department (AGFD) participated in the capture of desert bighorn sheep (*O. c. nelsoni*) in 6 geographic areas of Arizona; consistent with WAFWA guidelines, biological samples were collected for disease and health assessment. We compared the prevalence rates for infectious diseases in general, and for *M. ovipneumoniae* specifically, between the bighorn sheep populations in Arizona to evaluate the disease risk that might be associated with translocation.

Study Areas

We sampled desert bighorn sheep for disease prevalence in 6 areas in Arizona (Figure 1). Study areas included Kanab Creek in northern Arizona; Martinez Lake, Yuma Proving Grounds (YPG), and Kofa National Wildlife Refuge (KNWR) in southwestern Arizona; the Black Mountains in northwestern Arizona; and the Superstition Mountains in central Arizona (Figure 1).

Kanab Creek runs south from Utah to the Grand Canyon and water is readily available from permanent and perennial springs. The elevation ranges from 760 m to 1,650 m. The dominant vegetation types are the Intermountain Basins Mixed Salt Desert Scrub and Intermountain Basins Mixed Semi-desert Steppe (Arizona Game and Fish Department 2012). The mean winter high temperature is 8.5°C and the mean high summer temperature is 35.0°C (Western Regional Climate Center 2012). A seasonal pattern of cattle grazing occurs in some portions of the habitat at low stocking rates.

The KNWR, YPG, and Martinez Lake are in southwestern Arizona. Southwestern Arizona is composed of rugged mountain ranges separated by broad valleys. Elevation ranges from 400 m to 1,449 m on KNWR. The elevation range is somewhat broader for YPG and Martinez Lake with the lower limit at 20 m and peaks as high as 1,467 m. Annual precipitation across KNWR averages 17.7 cm (Western Regional Climate Center 2011). Annual average precipitation is lower at YPG and Martinez Lake (9.3 cm; Western Regional Climate Center 2011). Average daily high temperatures are 15.4°C in winter and 31.8°C in summer for KNWR; average daily high temps are 6.3°C in winter and 41.4°C in summer for YPG and Martinez Lake (Western Regional Climate Center 2011). Sonora-Mojave Creosotebush-White Bursage Desert Scrub and Sonoran Paloverde-Mixed Cacti are intermingled as the predominant vegetation types (Arizona Game Fish Department 2012). In the region, surface water is scarce except for the Colorado River; there are a few perennial springs, and ephemeral rock pools. A number of water catchments have been developed in the past 20 years. Areas of agricultural use are present along the Colorado River to the west and the Gila River to the south and east. Some domestic sheep grazing occurs on hay fields in the winter.

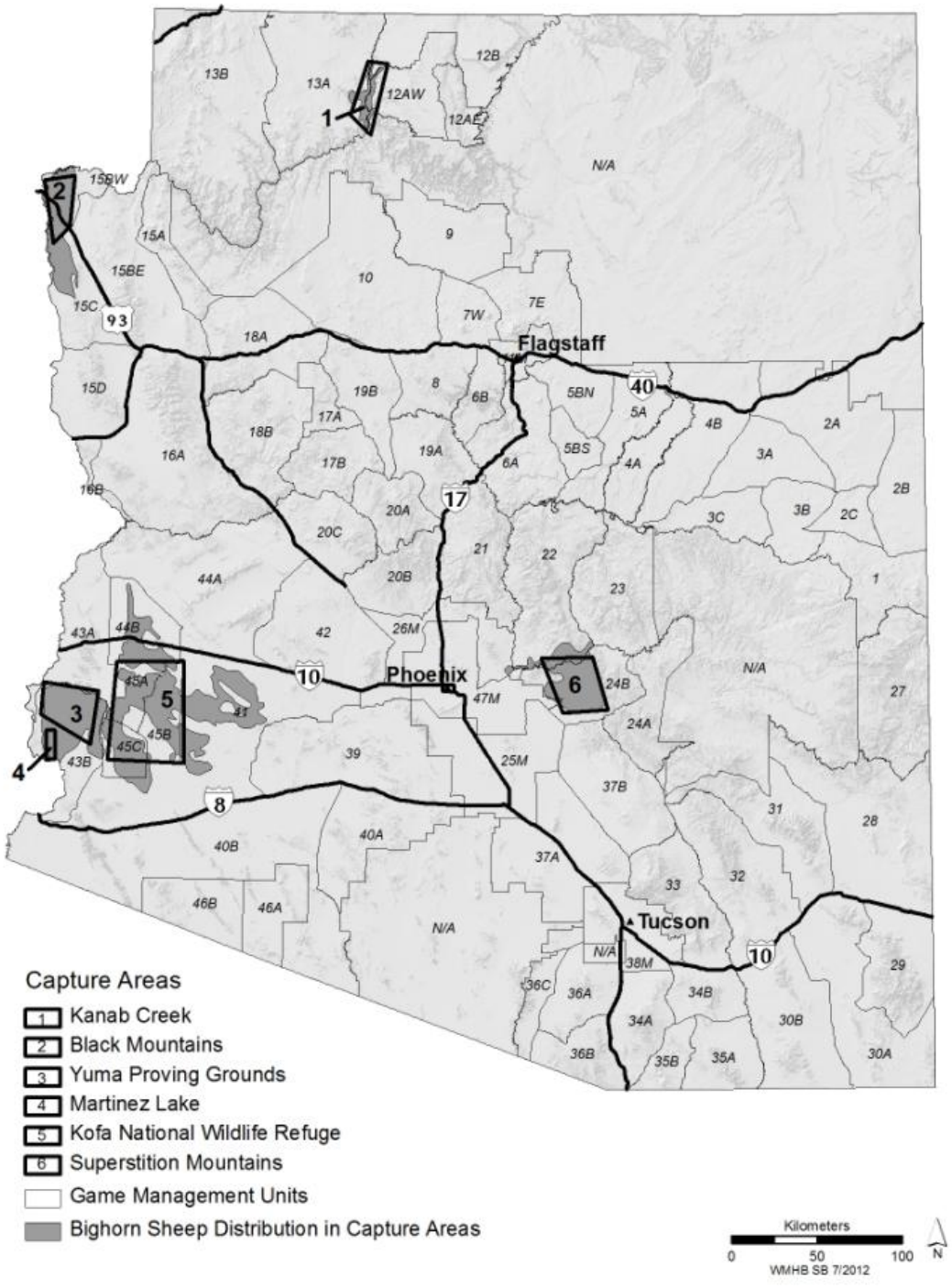

Figure 1. Locations of 6 populations of desert bighorn sheep tested for *Mycoplasma ovipneumoniae* in Arizona during 2009–2010.

The Black Mountains in northwestern Arizona range from 195 m to 1,510 m; the Colorado River flows along the western side of the range. The climate of the area is quite variable with the annual precipitation averaging 14 cm on the western side of the range compared to 27 cm on the eastern side (Western Regional Climate Center 2011). The mean high temperature is 17.6°C in January and 43.8°C in July near the Colorado River; the mean temperatures are slightly lower on the eastern side of the range (Western Regional Climate Center 2011). The vegetation communities consist of Sonora-Mohave Creosotebush-White Bursage in the lower elevations, Sonora Mid-elevation Desert Scrub on the western side of the range and Mohave mid-elevation mixed desert scrub on the eastern side of the range (Arizona Game Fish Department 2012).

The Superstition Mountains and adjacent Salt River Canyon have an average annual precipitation of 31 cm, a mean high of 15°C in winter, and a mean high of 40°C in the summer. The primary vegetation communities are Sonoran Paloverde-Mixed Cacti, Apacherian-Chihuahuan Mesquite Upland Scrub, and Mogollon Chaparral along a climbing elevation gradient (Arizona Game and Fish Department 2012). A domestic sheep driveway is present along the western edge of the bighorn sheep habitat.

Materials and Methods

One hundred twenty-four desert bighorn sheep were captured by helicopter with a net gun on 10 occasions from 6 different populations. From June 2009 to November 2012 we captured and sampled bighorn sheep in Kanab Creek (n = 11), YPG (n = 15), KNWR (n = 43), Martinez Lake (n = 12), and Superstition Mountains (n = 32). Eleven animals from the Black Mountains in the vicinity of U. S. Highway 93 were sampled during collar replacement efforts in November 2010.

Except for the bighorn sheep captured in the Black Mountains where bighorn sheep were processed at their capture site, all the animals were ferried by helicopter to a base station for processing after capture. Vital signs were monitored periodically during handling. When an animal's body temperature exceeded 40 °C, cold water and ice packs were applied externally, and subcutaneous or intravenous fluids were administered. No major wounds or injuries occurred during the captures; minor abrasions and lacerations were treated by cleaning with disinfectants and application of topical antibiotics. Two mortalities occurred during the capture in the Superstition Mountains.

We collected blood and swabs, either nasopharyngeal or oropharyngeal, from each animal; swabs were not collected from the Black Mountains, YPG populations, and 2 animals showing signs of contagious ecthyma in the Superstition Mountains. After collection, all samples were kept in insulated containers with gel ice packs. All swabs were shipped overnight to the Washington Animal Disease Diagnostic Laboratory (WADDL) on gel ice packs. Swabs were processed by first culturing in liquid media and then testing with polymerase chain reaction (PCR) to detect the presence of *M. ovipneumoniae* organisms (Besser et al. 2008, Weiser et al. 2012).

Blood was centrifuged and separated within 24 hours. Serum was either shipped within 48 hours or stored at -70°C until shipment overnight on gel ice packs. Detection of antibodies (positive or negative) to Infectious Bovine Rhinotracheitis (IBR), Bovine Respiratory Syncytial Virus (BRSV), and Parainfluenza 3 (PI3) was determined by virus neutralization, to Bluetongue Virus (BTV) was determined by competitive enzyme-

linked immunosorbent assay (cELISA), and to Epizootic Hemorrhagic Disease (EHD) was determined by agar gel immunodiffusion using National Animal Health Laboratory Network standard protocols. Detection of antibodies (positive, negative, or indeterminate) to *M. ovipneumoniae* was determined using a blocking enzyme-linked immunosorbent assay (WADDL 2011). Samples with less than 40% inhibition were considered negative. Samples with inhibition greater than or equal to 50% were considered positive. Samples with 40–50% inhibition were considered indeterminate by the laboratory and for the purposes of calculating seroprevalence were considered negative. In studies with domestic sheep and bighorn sheep, 50% inhibition of the ELISA by test sera represents the 95% confidence level for sheep (both domestic and bighorn sheep) defined as negative in the validation protocol.

The confidence interval for the seroprevalence rate of each population was calculated based on the normal approximation of a binomial distribution or based on the exact distribution depending on sample size (Thrusfield 2005:243). The size of the reference population for each area was based on survey data and population estimates made by the Arizona Game and Fish Department for each of the study areas. This estimate was then used to determine the probability of detecting disease given the size of the sample for the Superstition Mountains and Black Mountains (Thrusfield 2005). The χ^2 test was used to compare the seroprevalence rates between groups.

Results

Bighorn sheep populations in the Black and Superstition mountains did not possess antibodies for *M. ovipneumoniae* (Table 1). Because the Black Mountains support about 1,000 bighorn sheep and the Superstition Mountains support about 400, the seroprevalence could be no greater than 24% and 8.5%, respectively (Thrusfield 2005:240). The seroprevalence in the remaining 4 groups ranged from 58% (95% CI = 28–85%) testing positive in the YPG population to 82% (95% CI = 43–95%) testing positive in the Kanab Creek population (Table 1). The Kofa population seroprevalence in 2009 (76%, 95% CI = 63–89%) and in 2010 (57%, 95% CI = 18–90%) did not differ (P = 0.57, Table 2). In 3 of 4 populations tested, *M. ovipneumoniae* organisms were detected in swab samples (Table 1). Mycoplasma organisms were detected in 45% of the Kanab Creek bighorn sheep, the only population where oropharyngeal swabs were used. Nasal swabs yield 50–100% more positives than oropharyngeal swabs (Washington Animal Disease Diagnostic Laboratory 2011). The Superstition Mountains population tested negative on both titer and swab tests. The number of animals with positive swabs was about 43% in each of the seropositive populations (Table 1).

The 11 bighorn sheep tested from the Martinez Lake population and the 17 bighorn sheep tested from KNWR were positive for exposure to PI3 and BRSV (Table 3). The remaining populations varied in seroprevalence for BRSV from 87% in the YPG population to 28% in the Superstition Mountains population. A similar level of variability was seen for PI3 with 73% testing positive in the Kanab Creek population and 46% testing positive in the YPG population. No bighorn sheep tested positive for exposure to IBR. Two populations demonstrated exposure to EHD and BTV: Martinez Lake (8%) and Superstition Mountains (59%).

Discussion

Bighorn sheep populations in the western United States have been negatively

Table 1. Summary of Mycoplasma testing for desert bighorn sheep from 6 populations in Arizona, 2009–2010.

Population	Percent positive titers (*n* tested)	Percent positive swabs (*n* tested)
Martinez Lake	72.7 (11)	41.6 (12)
Yuma Proving Grounds	58.3 (12)	NT[a]
Kofa NWR 2009	75.6 (41)	NT
Kofa NWR 2010	57.1 (7)	42.8 (7)
Superstition Mountains	0 (32)	0 (30)
Kanab Creek	81.8 (11)	45.4 (11)[b]
Black Mountains	0 (11)	NT

[a] NT = Not tested
[b] Oral-pharyngeal swabs collected instead of nasal swabs. Nasal swabs yield 50–100% more positives than oral-pharyngeal swabs.

influenced by disease, habitat degradation, competition with livestock, and degradation of wildlife movement corridors since the late 1800s (Welsh and Bunch 1983, Jansen et al. 2006, Arizona Game and Fish Department 2009, Clifford 2009, Besser et al. 2012*b*). Psoroptic scabies, contagious ecthyma, pneumonia, and mycoplasma conjunctivitis have caused population decreases in desert bighorn sheep in Arizona (Welsh and Bunch 1983, Jansen et al. 2006, Arizona Game and Fish Department unpublished data). Population declines in bighorn sheep have been more frequently associated with outbreaks of pneumonia than any other disease syndrome (Clifford et al. 2009, Cassaigne et al. 2010, Besser et al. 2012b). Outbreaks of pneumonia in Rocky Mountain bighorn sheep (*O. c. canadensis*) have been well documented (Rudolph et al. 2007, Besser et al. 2008, Weiser et al. 2009, Wolfe et al. 2010). There are fewer records of pneumonia outbreaks in desert bighorn sheep (Cassaigne et al. 2010). In December 2005, coughing bighorn sheep were observed in the Kanab Creek area of Arizona; a subsequent assessment documented a population decline in association with the isolation of *Pasteurella* spp. and *M. ovipneumoniae*.

The initiation of a disease outbreak is the result of the interaction of host, pathogen, and environmental factors. Wehausen and Ramey (2011) described the process in 5 steps when discussing the probability of an outbreak occurring as the result of domestic sheep interacting with bighorn sheep. The same 5 steps could also describe the probability of an outbreak occurring as the result of a wandering bighorn sheep or translocation event. Yet, the probability of a wandering bighorn sheep carrying a pathogen to an adjacent bighorn sheep population is lower than the probability of domestic sheep carrying a pathogen (Clifford et al. 2009, Wehausen and Ramey 2011, Besser et al. 2012*a*). Also, the probability of translocated bighorn sheep carrying a pathogen could be markedly higher than that of a wandering bighorn sheep (Besser et al. 2012*b*). The probability of translocated animals transferring pathogens is also likely to be higher than for wandering animals because translocation can result in an increased shedding of pathogens (WAFWA 2009, Weiser et al. 2009). Our finding of 2 populations testing negative for exposure to *M. ovipneumoniae* provides justification for the WAFWA WHC recommendations that agencies routinely test both donor and recipient populations for bighorn sheep translocations.

The Arizona Game and Fish Department has used translocations since 1959 to augment existing populations and reestablish locally extirpated populations (Wakeling 2007). We chose to compare the prevalence of *M. ovipneumoniae* in bighorn

Table 2. Antibody titers to *M. ovipneumoniae* by year sampled in desert bighorn sheep from Kanab Creek and Kofa National Wildlife Refuge, Arizona.

Population	Year	*n* Tested	*n* Detected	% Detected
Kanab Creek	2006	11	10	91
	2009	11	9	82
Kofa National Wildlife Refuge	2001	23	6	26
	2009	41	31	76
	2010	7	4	58

Table 3. Summary of serology testing for viral diseases expressed as percentage of samples testing positive for desert bighorn sheep in 6 populations of Arizona, 2009–2010.

Population (*n* tested)	PI3	BRSV	IBR	EHD/BTV
Martinez Lake (11)	100	100	0	8.3
Yuma Proving Grounds (15)	46	86.7	NT[a]	0
Kofa National Wildlife Refuge (17)	100	100	0	0
Superstition Mountains (32)	65.6	28	0	59.4
Kanab Creek (11)	72.7	81.8	0	NT
Black Mountains (10)	70	30	0	0

[a] NT = not tested

sheep populations of Arizona because of the availability of a serological and PCR tests and because it had been identified in an outbreak in the Kanab Creek population. Mycoplasmas possess characteristics which allow a high rate of strain variability (Lysnyansky et al. 2001, Flitman-Tene et al. 2003). *M. ovipneumoniae* has been consistently isolated from bighorn sheep in outbreaks and while attempts to induce pneumonia by inoculating young bighorn sheep have been unsuccessful, mycoplasmas are known to depress the immune system and increase the severity of disease in the presence of other organisms (Razin et al. 1998, Besser et al. 2008, Dassanayake et al. 2010, Besser et al. 2012a).

The seroprevalence of *M. ovipneumoniae* varied from 0–80% in the tested populations. Organisms were also detected in populations which were seropositive; no organisms were recovered from populations which were seronegative. While the sample size for the Black Mountains population was small and seropositive animals could have been missed, the Superstition Mountains sample size is sufficient to conclude that the population has probably not been recently exposed to *M. ovipneumoniae.*

It is uncertain whether the presence of *M. ovipneumoniae* has contributed to significant morbidity or mortality in any of the desert bighorn sheep populations in Arizona. Signs of pneumonia and respiratory disease were reported in the Kanab Creek population in 2005 and subsequent testing found *M. ovipneumoniae* organisms in 8 of 11 bighorn sheep, a 73% prevalence rate, and pathogenic biovariants of *Mannheimia haemolytica* in 2 of 11 bighorn sheep (Arizona Game and Fish Department,

unpublished data). In 2009, this population continued to test positive for both of these organisms and seroprevalence remained high for *M. ovipneumoniae*. While no signs of respiratory disease have been noted, this population has not returned to the historical numbers found before the decline observed in 2003 (Arizona Game and Fish Department 2009). Two animals from the 2005 assessment were recaptured in 2009. One was seronegative in 2005 and the other was seropositive; in 2009, both were seropositve which suggests continued exposure to the organism. No *M. ovipneumoniae* organisms were detected in either animal in 2005 or 2009 which is not unexpected given the difficulty in recovering the organism with oropharyngeal swabs (Besser et al. 2008, Washington Animal Disease Diagnostic Laboratory 2011).

The KNWR population experienced a dramatic population decline between the 2000 and 2003 surveys. At about the same time, mountains lions (*Puma concolor*) were detected on the refuge. However, there was also an apparent increase in the number of animals testing positive for exposure to *M. ovipneumoniae* with 6 of 23 (26%, 95% CI = 8% to 44%) testing positive in 2001 and 26 of 33 testing positive in 2009 (79%, 95% CI = 63% to 89%) with the same ELISA test at the same diagnostic laboratory (the 2001 test was performed on banked serum, Table 2). Given that the sample sizes are comparable and of adequate size, this suggests an increase in the number of animals being exposed ($P \leq 0.0001$).

The results support the recommendations of the WAFWA WHC for the routine monitoring of populations for which translocation will be used as a management tool. One of the major limitations of this study is the small and inconsistent sample sizes, emphasizing the recommendation for statistically meaningful sample size for surveillance. However, the populations for which there is an adequate sample size also demonstrate a significant difference in exposure to *M. ovipneumoniae* based on both culture and serology. To confidently manage bighorn sheep populations with translocation, agencies should maintain statistically meaningful sample sizes; accurate population and demographic surveys are required to determine adequate sample sizes for disease monitoring. Natural animal movement, microbial strain shifts, and exposure to livestock could result in a change in the microflora of a population and agencies should incorporate regular testing of proposed donor and recipient populations in their management plans. Population declines have been documented in 2 of the bighorn sheep populations studied and we speculate exposure to this organism has contributed to the decline; in Kanab Creek, bighorn sheep were observed with signs of pneumonia and bronchopneumonia was found on gross necropsy of 1 animal.

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