DOI: 10.7589/JWD-D-20-00140

Journal of Wildlife Diseases, 57(3), 2021, pp. 579–589

ASSESSMENT OF DISEASE RISK ASSOCIATED WITH POTENTIAL REMOVAL OF ANTHROPOGENIC BARRIERS TO MOJAVE DESERT TORTOISE (*GOPHERUS AGASSIZII*) POPULATION CONNECTIVITY

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ABSTRACT: The Mojave Desert tortoise (*Gopherus agassizii*), federally listed as threatened, has suffered habitat loss and fragmentation due to human activities. Upper respiratory tract disease (URTD), a documented health threat to desert tortoises, has been detected at the Large-Scale Translocation Study Site (LSTS) in southwestern Nevada, US, a fenced recipient site for translocated animals. Our study aimed to 1) estimate prevalence of URTD and *Mycoplasma* infection at LSTS and three nearby unfenced sites; 2) assess whether *Mycoplasma* infection status was associated with developing clinical signs of URTD; and 3) determine whether such an association differed between LSTS and unfenced areas. We sampled 421 tortoises in 2016 to describe the current status of these populations. We evaluated three clinical signs of URTD (nasal discharge, ocular discharge, nasal erosions) and determined individual infection status for *Mycoplasma agassizii* and *Mycoplasma testudineum* by quantitative PCR and enzyme-linked immunosorbent assay. In 2016, LSTS had the highest prevalence of *M. agassizii* (25.0%; 33/132), *M. testudineum* (3.0%; 4/132), and URTD clinical signs (18.9%; 25/132). Controlling for other factors, clinical sign(s) were positively associated with *M. agassizii* infection (odds ratio [OR]=7.7, P=0.001), and this effect was similar among study sites (P>0.99). There was no association with *M. testudineum* status (P=0.360). Of the 196 tortoises in a longitudinal comparison of 2011–14 with 2016, an estimated 3.2% converted from *M. agassizii*-negative to positive during the study period, and incidence was greater at LSTS (P=0.002). Conversion to positive *M. agassizii* status was associated with increased incidence of clinical signs in subsequent years (OR=11.1, P=0.018). While *M. agassizii* and URTD are present outside the LSTS, there is a possibility that incidence of *Mycoplasma* infection and URTD would increase outside LSTS if these populations were to reconnect. Population-level significance of this risk appears low, and any risk must be evaluated against the potential long-term benefits to population viability through increased connectivity.

Key words: Disease risk assessment, *Gopherus agassizii*, Mojave Desert tortoise, *Mycoplasma agassizii*, translocation, upper respiratory tract disease.

INTRODUCTION

The Mojave Desert tortoise (*Gopherus agassizii*), a federally listed threatened species, occupies a variety of desert habitats in the southwestern US (USFWS 1994). Tortoise populations have declined throughout the Mojave Desert due largely to continued habitat loss, degradation, and other human activity (USFWS 2011; Allison and McLuckie 2018). As part of the management strategy for this imperiled species, displaced tortoises are often translocated to nearby suitable habitats to mitigate individual loss (Field et al. 2007; Nussear et al. 2012).

The Large-Scale Translocation Site (LSTS) is an approximately 100-km^2 area of Mojave Desert habitat in Ivanpah Valley in Clark County, Nevada, enclosed by either mountains or fencing on all sides. It served as a recipient site for displaced desert tortoises from the Las Vegas area between 1997 and 2014. Translocated tortoises included unwanted former pets, tortoises cleared from devel-

opment sites, and animals relinquished from other sources, creating a population of tortoises with mixed and diverse backgrounds and often unknown history (Field et al. 2007; Rideout 2015). Early translocations at this site included only rudimentary health evaluations prior to release, although individuals released after 2009 were subject to rigorous health screenings (USFWS 2015). This isolated population of tortoises is currently (2020) estimated to consist of fewer than 400 adults and an unknown number of juvenile tortoises (Scott et al. 2020), representing an effective population size that might be sufficient to minimize short-term but not long-term extirpation risk (Soulé 1980).

In the surrounding Ivanpah Valley, the desert has been heavily utilized for human infrastructure, including Interstate Highway 15, several large solar energy installations, and casinos, among other developments. These developments fragment tortoise habitat and, with the fenced LSTS and Interstate Highway 15 severing the western half of the valley, reduce the overall size of the tortoise population. Instead of one functional, interconnected population within the valley, the LSTS contributes to the formation of multiple smaller patches with fewer tortoises. Smaller, disjunct patches have elevated extinction probabilities, increasing the risk that persistence within any particular patch will decrease (Fahrig 2002; Ovaskainen et al. 2002). One management option under consideration to improve the resilience of the tortoise population in Ivanpah Valley is to create routes of passage through the human-created barriers between the LSTS and surrounding Ivanpah Valley, restoring the ability of patches to be recolonized across the LSTS in the event of local population declines. It is important to assess infectious disease transmission risks that may be posed by this management action (Aiello et al. 2014).

The most serious infectious disease concerns in Mojave Desert tortoises relate to upper respiratory tract disease (Jacobson et al. 1991; Brown et al. 1994; Rideout 2015). The cause of this disease syndrome is not thoroughly understood, but two species of *Mycoplasma* (*M. agassizii* and *M. testudineum*) have been detected in affected animals and are believed to play an important role in this disease (Jacobson et al. 1991; Brown et al. 1994). Testudinid herpesviruses have also been detected in tortoises with oropharyngeal and respiratory disease (Harper et al. 1982; Pettan-Brewer et al. 1996), but the pathologic significance of these viruses (in particular of TeHV2) in desert tortoises is not known (Jacobson et al. 2012).

We evaluated the prevalence of clinical signs of upper respiratory disease and *Mycoplasma* infection status in tortoises both within the LSTS and outside the LSTS in neighboring areas of the Ivanpah Valley. We also aimed to determine whether there is an association between *Mycoplasma* infection status and clinical signs and if any association differs between the LSTS and neighboring areas. Our findings will inform future decisions regarding management of animals within the LSTS and conservation of the Ivanpah Valley population.

MATERIALS AND METHODS

Study population

The study population (Table 1) included 421 desert tortoises that had health assessments between March and October 2016 at one of four different study sites (Fig. 1) in the Ivanpah Valley along the California-Nevada border, southwestern US. The study sites were the LSTS (Nevada, 35°44′N, 115°23′W), Ivanpah Solar Electric Generating System (ISEGS, California, 35°35′N, 115°29′W), Desert Stateline Solar Facility (California, 35°36′N, 115°26′W), and Silver State South Solar Energy Center (Nevada, 35°38′N, 115°21′W). In addition to translocated tortoise presence at LSTS, all solar sites included tortoises that had been translocated out of the development footprint and into the adjacent habitat. The health assessments included an evaluation of clinical signs for upper respiratory disease (further described in the upcoming text) coupled with samples of plasma and oral swabs or nasal flushes. All animals were individually identified, and the origin of the animal as a translocatee or a preexisting resident animal was noted. A subset of the tortoises (33 from LSTS and 163 from ISEGS) were included in the longitudinal study at two time points (2011–14, and 2016) to evaluate

TABLE 1. Characteristics of the study population of 421 Mojave Desert tortoises (*Gopherus agassizii*) from the cross-sectional study. Animals included in the longitudinal study (Table 2) were drawn from this set. The data were collected at health assessments conducted between 2011 and 2016 at four study sites: Large-Scale Translocation Site (LSTS; Nevada, USA), Ivanpah Solar Electric Generating System (ISEGS; California, USA), Desert Stateline Solar Facility (California, USA), and Silver State South Solar Energy Center (Nevada, USA).

	ISEGS	LSTS	Silver State	Stateline	Total
Sex					
Female	66	33	5	15	119
Male	81	90	11	20	202
Unknown	91	9	0	0	100
Age class[a]					
Adult	139	111	16	33	299
Immature	82	21	0	2	105
Juvenile	17	0	0	0	17
Total	238	132	16	35	421

[a] Age classes based on straight midline carapace length (MCL). Adult = ≥200-mm MCL; immature = 100–199-mm MCL; juvenile = <100-mm MCL.

associations between pathogens and clinical signs over time.

Data collection and sample testing

Clinical signs were documented during health assessments conducted by trained field biologists according to established protocols (USFWS 2015, 2016). Health assessments were conducted during 2011–14 prior to release of animals at LSTS and in 2016 for this study. Biologists conducted annual to biannual health assessments at the solar sites in accordance with each project's monitoring plans. Blood was collected from the subcarapacial vein or brachial plexus, kept chilled in the field, and plasma was separated and stored at ≤−70 C. Plasma was tested for *M. agassizii* and *M. testudineum* antibodies by a previously described enzyme-linked immunosorbent assay (ELISA) (Wendland et al. 2007; Jacobson and Berry 2012) at the University of Florida *Mycoplasma* Testing Lab. Positive test results on the ELISA were interpreted as positive infection (rather than merely exposure status), as this test has been validated in desert tortoises against a standard of infection, and infection appears to be either lifelong or of very long duration (Jacobson et al. 1991; Sandmeier et al. 2017). Oral swab or nasal flush samples were tested at the Molecular Diagnostics Laboratory, San Diego Zoo Global,

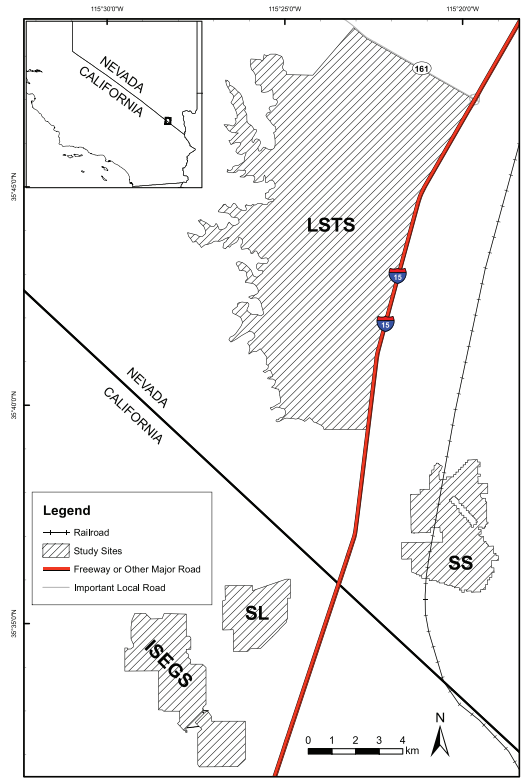

FIGURE 1. Map of the study sites. LSTS=Large-Scale Translocation Study Site (Nevada, USA), ISEGS=Ivanpah Solar Electric Generating System (California, USA), SL=Desert Stateline Solar Facility (California, USA), and SS=Silver State South Solar Energy Center (Nevada, USA). The straight, linear boundaries on the northern, eastern, and southern boundaries of LSTS are fenced to prevent movement of Mojave Desert tortoises (*Gopherus agassizii*). The convoluted western boundary of the LSTS is not fenced, but rather is defined by the 1,250-m elevation contour.

for *M. agassizii*, *M. testudineum*, and TeHV2 using previously described quantitative (q)PCR assays (Braun et al. 2014). For our study, results of qPCR and ELISA tests were interpreted in parallel: an animal that was positive on either test was considered to be infected.

Study design, risk factors, and statistical analyses

Cross-sectional study: Using the entire study population (n=421), we conducted a cross-sectional study examining the association between pathogen status and health during survey year 2016. The primary outcome of interest was 'presence of any clinical sign' associated with upper respiratory tract infection that was docu-

TABLE 2. Sample size and source populations from a total study population of 421 Mojave Desert tortoises (*Gopherus agassizii*) used in each analysis. The study population for the longitudinal study (n=196) is a subset of that use in the cross-sectional study (n=421), restricted to only those animals with data at both the initial (2011–14) and subsequent (2016) health assessment (HA). The four number rows (1–4) are independent subsets used for analyses, restricted according to the specified eligibility restrictions (see Materials and Methods for further details). To improve comparability, aims ii–iv in the longitudinal analyses used frequency matching to balance (and rebalance as needed) the distribution of initial sampling year between the two study sites in a 1:5 ratio (LSTS:ISEGS). The data were collected at HAs conducted between 2011 and 2016 at four study sites: Large-Scale Translocation Site (LSTS; Nevada, USA), Ivanpah Solar Electric Generating System (ISEGS; California, USA), Desert Stateline Solar Facility (California, USA), and Silver State South Solar Energy Center (Nevada, USA).

Aim	Eligibility criteria	Sample size (n)
Cross-sectional study	Entire study population	421
Longitudinal study	Data from LSTS and ISEGS at both initial and 2016 HA	196
1) Crude incidence risk	Free of *Mycoplasma* infection at initial HA	185 (*Mycoplasma agassizii*) 192 (*M. testudineum*)
2) Comparison of incidence risk between study sites	Free of *Mycoplasma* infection at initial HA	143 (*M. agassizii*) 186 (*M. testudineum*)
3) Infection at initial HA → clinical signs at subsequent HA	Free of clinical signs at initial HA	156
4) Incidence of new infection → clinical signs at subsequent HA	Free of both clinical signs and *Mycoplasma* infection at initial HA	130 (*M. agassizii*) 156 (*M. testudineum*)

mented during a health assessment, including nasal discharge, nasal erosions, and ocular discharge. The primary risk factors were evidence of infection with either *M. agassizii*, *M. testudineum*, or TeHV2. We fit separate univariate logistic regression models to examine the crude associations between the hypothesized predictors and the presence of clinical signs. We then fit multivariate logistic regression models to the data to adjust associations between infection and clinical signs for potential confounders, other covariates, and to evaluate effect modification. We adjusted all multivariate models for sex (male, female, unknown), midline carapace length, study site (four sites, see above for details), and translocation status (translocated vs. resident).

Longitudinal study: We conducted longitudinal analyses among subsets of eligible animals from inside the fence at the LSTS and outside the fence at ISEGS with health data available at two time points. This included data from the initial (2011–14) and subsequent (2016) health assessments. Sample size varied for different analyses (Table 2). We addressed four aims in the longitudinal study to:

1) Estimate incidence risk of *Mycoplasma* infection during the study period performed on all animals free of *Mycoplasma* infection at the initial health assessment (i.e., the subset of tortoises at risk of becoming infected during the study period).
2) Compare incidence risk of *Mycoplasma* infection between study sites. To account for the variation in initial sampling year and follow-up times, we selected a subset of tortoises free of *Mycoplasma* infection at the initial health assessment from the two sites, ISEGS and LSTS. For each eligible tortoise from LSTS (inside the fence), five tortoises from ISEGS (outside the fence) were randomly selected to reflect the same distribution of initial health assessment year (and corresponding follow-up times) that were observed within the LSTS. This frequency-matching approach (Dohoo et al. 2003) helped to control for potential confounding and unevenness in the data across sampling years and locations. A ratio of 5:1 was used, as this was the greatest number of matched controls that could be drawn from the available ISEGS sample population and thus should be adequate to maximize statistical power (Rothman et al. 2008).
3) Assess the association between *Mycoplasma* infection status at the initial health assessment and the presence of clinical signs of URTD at the 2016 health assessment. We used a frequency-matched cohort selected in the same manner as described in Aim 2, but drawn from an initial pool containing only animals free of clinical signs at initial assessment (i.e., at risk of developing clinical signs during the study period).

4) Assess the association between conversion from *Mycoplasma*-negative to positive between the two health assessments and the presence of URTD clinical signs at the 2016 health assessment. The same frequency-matched cohort approach was applied, drawn from the set of animals free of both clinical signs and *Mycoplasma* infection at the initial assessment.

Clinical signs and other predictors were assessed as described in the cross-sectional study, and we used the same covariates in the models. We fit separate logistic regression models for each outcome. Models included one primary risk factor, and we adjusted all models for the year of initial health assessment (the variable on which the match was based). All statistical analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Cross-sectional study

Overall, 43 of 421 (10.2%) tortoises had one or more putative clinical signs of URTD at the 2016 health assessment (Table 3). Prevalence of the various clinical signs varied widely across the study sites, the most frequent being nasal erosions (9.3%; 39/421). Tortoises at LSTS showed the highest prevalence of clinical signs (18.9%; 25/132), with all other sites having prevalence estimates less than 7%.

At the 2016 health assessment, *M. agassizii* infection ranged from 0.0% (0/16) at Silver State to 25.0% (33/132) at LSTS (Table 3). We only detected *M. testudineum* at ISEGS (0.4%; 1/238) and LSTS (3.0%; 4/132). We only identified TeHV2 in a single animal from the LSTS, so this pathogen was not further evaluated. The ELISA returned more positive results than did the qPCR (Table 3). Only three of the 14 qPCR-positive animals were ELISA-negative, whereas a majority of ELISA-positive animals (21/32) were PCR-negative.

Mycoplasma agassizii infection was significantly associated (Table 4) with presence of clinical signs of URTD (odds ratio [OR]=6.22; 95% confidence interval [CI]=2.40–16.10). The odds of clinical signs increased with increasing size of the tortoise as measured by the midline carapace length (OR=1.12 per 10 mm; 95% CI=0.99–1.27). The only variables associated with presence of clinical signs in the final multivariate model predicting *M. testudineum* were increasing size (midline carapace length OR=1.14 per 10 mm; 95% CI=1.01–1.28) and site (LSTS study site OR=2.34; 95% CI=1.14–4.77); infection status was not a significant predictor of the presence of clinical signs in this model (OR=2.41; 95% CI=0.36–15.94). Presence of clinical signs was not associated with sex or translocation status in either model (*M. agassizii* or *M. testudineum*), and these results did not differ between tortoises on either side of the barrier fencing (i.e., inside vs. outside LSTS; interaction $P>0.05$).

TABLE 3. Prevalence (*n*) of clinical signs of upper respiratory disease and test results for *Mycoplasma agassizii* and *Mycoplasma testudineum* among 421 Mojave Desert tortoises (*Gopherus agassizii*). The data were collected at health assessments conducted between 2011 and 2016 at four study sites: Large-Scale Translocation Site (LSTS; Nevada, USA), Ivanpah Solar Electric Generating System (ISEGS; California, USA), Desert Stateline Solar Facility (California, USA), and Silver State South Solar Energy Center (Nevada, USA).[a]

	ISEGS	LSTS	Silver State	Stateline	Total
Clinical signs					
Nasal discharge	0	8	0	0	8
Nasal erosions	16	21	1	1	39
Ocular discharge	0	5	0	0	5
Any clinical sign	16	25	1	1	43
M. agassizii					
ELISA-positive	1	30	0	1	32
qPCR-positive	0	14	0	0	14
Either method	1	33	0	1	35
M. testudineum					
ELISA-positive	1	0	0	0	1
qPCR-positive	0	4	0	0	4
Either method	1	4	0	0	5
Total	238	132	16	35	421

[a] ELISA = enzyme-linked immunosorbent assay; qPCR = quantitative PCR.

Longitudinal study

Six tortoises converted from *M. agassizii*-negative to positive between the initial sampling year (2011–14) and 2016, for an

TABLE 4. Multivariate logistic regression model predicting presence of any clinical signs of upper respiratory tract disease (see Table 3 for the list of clinical signs) among 421 Mojave Desert tortoises (*Gopherus agassizii*). The data were collected at health assessments conducted between 2011 and 2016 at four study sites: Large-Scale Translocation Site (LSTS; Nevada, USA), Ivanpah Solar Electric Generating System (ISEGS; California, USA), Desert Stateline Solar Facility (California, USA), and Silver State South Solar Energy Center (Nevada, USA). *Mycoplasma agassizii* status was determined by a parallel interpretation of the enzyme-linked immunosorbent assay and quantitative PCR tests (see Table 3 for test results). The first row for each variable denotes the baseline or reference level of the covariate, for which the odds ratio (OR) is 1.0 by definition.[a]

Variable	Level	OR	*P* value[b]	95% CI
M. agassizii	Negative	1.00	REF	—
	Positive	6.22	0.0002	2.40–16.10
Study site	ISEGS	1.00	REF	—
	LSTS	1.33	0.4965	0.58–3.05
	Silver State	0.82	0.8544	0.10–6.85
	Stateline	0.26	0.2163	0.03–2.20
Sex	Female	1.00	REF	—
	Male	2.32	0.1117	0.83–6.01
	Unknown	4.67	0.1120	0.70–31.20
Midline carapace length	Per 10 mm	1.12	0.0799	0.99–1.27
Translocation status	Translocated	1.00	REF	—
	Resident	1.61	0.2041	0.77–3.34

[a] — = not applicable; 95% CI = 95% confidence interval; REF = reference level.

[b] *P* values calculated by the Wald method.

estimated incidence of 3.2% (6/185). The incidence of infection was greater inside the fence (22.7%; 5/22) in the LSTS compared with outside (0.6%; 1/163) at ISEGS (Fisher's exact test for difference in proportions $P<0.0001$). Just two tortoises converted from being *M. testudineum*-negative to positive, with an estimated incidence of 1.0% (2/192). The *M. testudineum* incidence inside the fence within the LSTS was 3.2% (1/31) and outside the fence at ISEGS was 0.6% 1/161). These proportions were not significantly different ($P=0.296$).

There was no significant association ($P=0.418$) between *M. agassizii* infection status at the initial health assessment and presence of URTD clinical signs at the 2016 health assessment. However, among the subset of tortoises that were apparently healthy at the initial health assessment (i.e., no clinical signs and no evidence of infection), the odds of having clinical signs in 2016 were significantly greater (Table 5) for tortoises that converted to *M. agassizii*-positive status during the study period compared to those that did not (OR=11.61; 95% CI=2.11–63.84). This OR should be interpreted with caution, due to the very small case count ($n=6$), but the available data do suggest that the true value likely exceeds one. For the same reason, it was not possible to fit a multivariable model adjusted for all potential confounders and secondary risk factors as in the cross-sectional study. Due to the very small number of cases of conversion ($n=2$), data for *M. testudineum* were not analyzed in this way.

As in the cross-sectional study, we evaluated possible differences in these associations inside the fence compared with outside by the addition of interaction terms into each model. We found no evidence of effect modification for this association between change in *M. agassizii* infection status and URTD clinical signs; all *P* values for interaction terms were much greater than 0.05.

DISCUSSION

Prevalence of *M. agassizii* was highest in the LSTS, and we found evidence that the risk

TABLE 5. Logistic regression models (two separate models) predicting presence of any putative clinical sign of upper respiratory tract disease among a frequency-matched cohort of Mojave Desert tortoises (*Gopherus agassizii*). Model 1: *n*=156, Model 2: *n*=130. Data were collected at initial health assessment (HA) between 2011–14 and at a subsequent HA in 2016 at two study sites: Large-Scale Translocation Site (LSTS; Nevada, USA) and Ivanpah Solar Electric Generating System (ISEGS; California, USA). Both models are adjusted for year of first sampling. *Mycoplasma agassizii* status based on parallel interpretation of species-specific quantitative PCR and enzyme-linked immunosorbent assay tests (see Materials and Methods for details). *Mycoplasma agassizii* conversion refers to change from *Mycoplasma*-negative status to *Mycoplasma*-positive status during the study period.[a]

Variable	Level	OR	*P* value[b]	95% CI
M. agassizii at initial HA	Negative	1.00	REF	—
	Positive	2.57	0.4177	0.26–25.25
M. agassizii conversion	No	1.00	REF	—
	Yes	12.50	0.0053	2.11–74.01

[a] — = not applicable; 95% CI = 95% confidence interval; OR = odds ratio.

[a] *P* values calculated by the Wald method.

of conversion from *Mycoplasma*-negative to positive was higher inside the fenced LSTS area than outside at ISEGS during the study period. As such, if the fence is made permeable and tortoise populations are allowed to mix, it is reasonable to expect some increased risk of transmission in surrounding areas. Although the fence itself is not a barrier to URTD transmission, increased movement of infected animals across the boundary could lead to an increase in the contact rate between infected and naïve individuals.

Mycoplasma infection is not, however, restricted to the LSTS at present; infection has long been documented in tortoises outside the fence and in other populations throughout their range. Evidence of *M. agassizii* infection was detected (based on ELISA-positives) at two of three sites outside the fence at varying seroprevalence. The presence of seropositive tortoises indicates that they have been previously exposed and may still be infected, assuming infection is of long duration, as is generally believed (Jacobson et al. 1991; Sandmeier et al. 2017). Clinical signs consistent with URTD were also documented in tortoises outside of the fence. In 2016, qPCR-positive *M. agassizii* and *M. testudineum* infections were identified only inside the LSTS, but one case of seroconversion from negative to positive status was documented outside the fence during our study period. This suggests that transmission is ongoing outside the fenced area, albeit probably with lower frequency than inside. Previous work has also detected *M. agassizii* across much of the distribution of the Mojave Desert tortoise; infection prevalence based on qPCR was estimated at 56.3% in another surveyed population in the Ivanpah Valley in 2011–12 (Weitzman et al. 2017). We found associations between positive *M. agassizii* infection status and presence of clinical signs of URTD, particularly in animals that had converted from negative to positive *Mycoplasma* status between initial and subsequent health assessments. This finding is consistent with previous work identifying this pathogen as a suspected cause of URTD in desert tortoises (Jacobson et al. 1991; Brown et al. 1994). To address the question of whether LSTS animals manifest URTD differently, perhaps either due to genetic differences related to their provenance or due to other concurrent infections, we compared the association between *M. agassizii* infection and URTD signs in LSTS animals with that seen in animals in the three study sites outside the fence. We did not find evidence that this association varies inside the fence compared with outside.

We did not have adequate data to directly assess the implications of either URTD or *Mycoplasma* infection for long-term survival, but our dataset did include animals that

survived the entire study period, potentially continuing to reproduce and contribute to genetic diversity and connectivity in this area (Dutcher et al. 2020). To understand the true impact of *M. agassizii* on tortoise populations, long-term monitoring of the same individuals over time will be needed. This could mean prospectively following large numbers of tortoises, with and without this infection, to determine whether there are differences in survival or reproduction that could negatively impact population growth or viability.

Long-term monitoring may also help refine our knowledge of the constellation of clinical signs that are the best predictors of true infection status. For example, ocular swelling was observed frequently among tortoises that tested negative for *Mycoplasma*, as seen in populations of tortoises at Silver State and Stateline, and may not be closely associated with *Mycoplasma* infection status. Prospective evaluation of clinical signs and how they correlate with infection status will increase our understanding of the disease course and improve the ability to monitor indicators of health status in individuals and populations.

Our study did not distinguish between different stages of infection (e.g., very recently infected animals from those that have been infected for a long period) and, by interpreting the qPCR and ELISA tests in parallel, it is assumed that both reflect infection. A parallel interpretation results in a composite diagnostic with enhanced sensitivity, at some cost to specificity (Dohoo et al. 2003). Both the ELISA (Wendland et al. 2007) and the qPCR (Braun et al. 2014) tests we used have high specificity, and the reduction in specificity approaches zero as individual test specificity approaches 100%. Long-term field studies have shown that desert tortoises infected with *M. agassizii* may take up to 2 yr to develop a positive ELISA result (Aiello et al. 2019; Drake et al. 2019), meaning that the achieved sensitivity of the ELISA in a free-ranging population with a range of infection stages may well be lower. Intermittent shedding and declining shedding over time (Aiello et al. 2019) may impair the real-world sensitivity of qPCR testing. Both diagnostic tests, therefore, probably have high specificity and imperfect (though uncertain) sensitivity when used in free-ranging populations with mixed stages of infection, making a parallel interpretation the most desirable approach to optimize the use of available diagnostic testing data for the purposes of this study.

Estimating the true risk of transmission depends on the number of susceptible and infectious animals in the population and the contact rates between them. Differences in contact rates over space and time may in turn be driven by host population density and by heterogeneity in host behavior and shedding. The roles of population density and host contact rates in driving *Mycoplasma* transmission have not been assessed in free-ranging tortoises, but studies of captive animals indicate that host contact rates may be both variable and significant in driving *Mycoplasma* transmission risk (Aiello et al. 2016). The present data alone are insufficient to quantify the risk and the potential long-term impact of these drivers of transmission dynamics. Factors that may further increase the risk of disease transmission, but which were not evaluated in our study, include variation in *Mycoplasma* infection load among infected animals, existence of persistent severe infections, and changes in contact frequency or duration between naïve and infected animals (Aiello et al. 2016). While those authors describe predominantly short interactions between tortoises in their study, for which models predicted a low transmission probability, they do caution about the risk posed by a few "super-spreaders" (Aiello et al. 2016).

Stress and disturbance of behavior and social structures have been linked to increased shedding of infectious organisms in wildlife hosts (Woodroffe et al. 2009; Jacobson et al. 2014). It is possible that adding safe passages through the fence and under highways may result in fewer disturbances to habitat use, animal movement, and contact rates than would translocation. If this is true, we would expect a smaller effect via stress-induced shedding and excessive movement of tortoises beyond their present home ranges compared with a translocation event, but only if the

populations are at or near equilibrium and population densities do not differ greatly across the boundary.

The risk of increased disease transmission associated with opening fences to restore connectivity in the Ivanpah Valley is determined by the amount of animal movement through the created openings and the *M. agassizii* infection prevalence among those animals. Adult desert tortoises rarely disperse from established home ranges. For example, two relatively long-term studies (106 and 253 tortoise-years respectively) of desert tortoises found very low rates of long-distance movement by adult tortoises, with movements in excess of 1 km from established home ranges very rare (Vamstad et al. 2013; Averill-Murray et al. 2020). Although recently translocated adult tortoises have been known to make long exploratory movements, translocated desert tortoises typically establish home ranges comparable to residents within 2 yr (Field et al. 2007; Nussear et al. 2012), so we expect all tortoises in this study to show fidelity to established home ranges as residents.

We therefore anticipate that on an annual basis, few tortoises would diffuse into and out of the LSTS; that initially these dispersers will come from among those currently residing closest to the fenced borders; and that nonreproductive tortoises will be most represented among dispersers. Over many years, it will become more likely that tortoises farther from the fences and closer to the interior of the LSTS will be represented by their descendants among the dispersers; not all tortoises currently alive in the LSTS will encounter the passages to the outside but, at a population level, this will be sufficient to keep tortoises inside and outside the LSTS within the same functional population. We found a prevalence of *M. agassizii* of 25% (95% CI=17.9–33.3) within the LSTS. Assuming that our sampled population is representative and all tortoises are equally likely to disperse, this would also be the approximate expected probability that a disperser from the LSTS is infected. Most dispersing tortoises are juveniles (Averill-Murray et al. 2020) and our study, in agreement with previous work at other sites (Jacobson et al. 2014), found that smaller tortoises were less likely than larger ones to be infected by both *M. agassizii* and *M. testudineum*. *Mycoplasma* transmission is expected to require close contact (Brown et al. 2002; Aiello et al. 2016), which in tortoises would usually involve courtship, mating, fighting, and sharing of burrows by adults and is consistent with the lower prevalence in smaller tortoises in our study. Nonetheless, it remains possible that incidence of *Mycoplasma* infection and/or URTD in tortoises outside the fence may still increase for a period of time, should connectivity be restored between LSTS and the surrounding Ivanpah Valley.

We found that: 1) both *M. agassizii* infection and clinical signs of URTD are present inside and outside the LSTS, 2) both *M. agassizii* infection and clinical signs of URTD were detected more frequently inside the LSTS, and 3) *M. agassizii* prevalence and incidence of new infections were both associated with clinical signs of URTD; hence this pathogen is probably a greater concern as a suspected cause of URTD in this population than are other pathogens we evaluated. A decision to allow passage through the fence at LSTS carries some risk of increased disease transmission and is contentious for this reason (R.C.A.-M. pers. obs.). This risk must be weighed against the potential benefits of enhanced connectivity and population viability of desert tortoises in Ivanpah Valley (Scott et al. 2020). If the LSTS remains isolated, eventual extirpation within the site is possible due to its small population (Newman and Pilson 1997). Above all, the concern of URTD in desert tortoises and the conflict between expanding urban and energy development in the southwestern US are contentious issues. Effective risk communication will be of paramount importance in achieving an acceptable management outcome for stakeholder groups.

ACKNOWLEDGMENTS

This study was supported by the US Bureau of Land Management, US Fish and Wildlife Service (USFWS), and San Diego Zoo Global. Health

assessment sampling activities were conducted within the LSTS under USFWS recovery permit TE-108507-2 by the Great Basin Institute, solar project sites by numerous field biologists under USFWS Biological Opinions 8-8-10-F-24, 8-8-13-F-43, and 84320-2010-F-0208-R003 and within the Silver State location in 2016 under USFWS recovery permit TE-030659 by the US Geological Survey (USGS). We thank the USGS, University of Nevada-Reno, and University of California, Los Angeles for sharing data and archived samples and Lori Scott for data management assistance. We thank Jennifer Burchell, Asa Preston, Jamie Stoll, and the many field staff for their dedication to collecting data for this project. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service, US Geological Survey, or San Diego Zoo Global.

LITERATURE CITED

Aiello CM, Esque TC, Nussear KE, Emblidge PG, Hudson PJ. 2019. The slow dynamics of mycoplasma infections in a tortoise host reveal heterogeneity pertinent to pathogen transmission and monitoring. *Epidemiol Infect* 147:e12.

Aiello CM, Nussear KE, Esque TC, Emblidge PG, Sah P, Bansal S, Hudson PJ. 2016. Host contact and shedding patterns clarify variation in pathogen exposure and transmission in threatened tortoise *Gopherus agassizii*: Implications for disease modelling and management. *J Anim Ecol* 85:829–842.

Aiello CM, Nussear KE, Walde AD, Esque TC, Emblidge PG, Sah P, Bansal S, Hudson PJ. 2014. Disease dynamics during wildlife translocations: Disruptions to the host population and potential consequences for transmission in desert tortoise contact networks. *Anim Conserv* 17:27–39.

Allison LJ, McLuckie AM. 2018. Population trends in Mojave Desert tortoises (*Gopherus agassizii*). *Herpetol Conserv Biol* 13:433–452.

Averill-Murray RC, Fleming CH, Riedle JD. 2020. Reptile home ranges revisited: A case study of space use of Sonoran Desert tortoises (*Gopherus morafkai*). *Herpetol Conserv Biol* 15:253–271.

Braun J, Schrenzel M, Witte C, Gokool L, Burchell J, Rideout BA. 2014. Molecular methods to detect *Mycoplasma* spp. and Testudinid herpesvirus 2 in desert tortoises (*Gopherus agassizii*) and implications for disease management. *J Wildl Dis* 50:757–766.

Brown DR, Schumacher IM, Mclaughlin GS, Wendland LD, Mary EB. 2002. Application of diagnostic tests for mycoplasmal infections of desert and gopher tortoises with management recommendations. *Chelonian Conserv Biol* 4:497–507.

Brown MB, Schumacher IM, Klein PA, Keith H, Correll T, Jacobson ER. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infect Immun* 62:4580–4586.

Dohoo I, Martin W, Stryhn H. 2003. *Veterinary epidemiologic research*. AVC Inc., Charlottetown, Prince Edward Island, Canada, 706 pp.

Drake KK, Aiello CM, Bowen L, Lewison RL, Esque TC, Nussear KE, Waters SC, Hudson PJ. 2019. Complex immune responses and molecular reactions to pathogens and disease in a desert reptile (*Gopherus agassizii*). *Ecol Evol* 9:2516–2534.

Dutcher KE, Vandergast AG, Esque TC, Mitelberg A, Matocq MD, Heaton JS, Nussear KE. 2020. Genes in space: What Mojave Desert tortoise genetics can tell us about landscape connectivity. *Conserv Genet* 21:1–15.

Fahrig L. 2002. Effect of habitat fragmentation on the extinction threshold: A synthesis. *Ecol Appl* 12:346–353.

Field KJ, Tracy CR, Medica PA, Marlow RW, Corn PS. 2007. Return to the wild: Translocation as a tool in conservation of the desert tortoise (*Gopherus agassizii*). *Biol Conserv* 136:232–245.

Harper PAW, Hammond DC, Heuschele WP. 1982. A herpesvirus-like agent associated with a pharyngeal abscess in a desert tortoise. *J Wildl Dis* 18:491–494.

Jacobson ER, Berry KH. 2012. *Mycoplasma testudineum* in free-ranging desert tortoises, *Gopherus agassizii*. *J Wildl Dis* 48:1063–1068.

Jacobson ER, Berry KH, Wellehan JFX, Origgi F, Childress AL, Braun J, Schrenzel M, Yee J, Rideout B. 2012. Serologic and molecular evidence for Testudinid herpesvirus 2 infection in wild Agassiz's desert tortoises, *Gopherus agassizii*. *J Wildl Dis* 48: 747–757.

Jacobson ER, Brown MB, Wendland LD, Brown DR, Klein PA, Christopher MM, Berry KH. 2014. Mycoplasmosis and upper respiratory tract disease of tortoises: A review and update. *Vet J* 201:257–264.

Jacobson ER, Gaskin JM, Brown MB, Harris RK, Gardiner CH, LaPointe JL, Adams HP, Reggiardo C. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). *J Wildl Dis* 27:296–316.

Newman D, Pilson D. 1997. Increased probability of extinction due to decreased genetic effective population size: Experimental populations of *Clarkia pulchella*. *Evolution* 51:354–362.

Nussear KE, Tracy CR, Medica PA, Wilson DS, Marlow RW, Corn PS. 2012. Translocation as a conservation tool for Agassiz's desert tortoises: Survivorship, reproduction, and movements. *J Wildl Manage* 76: 1341–1353.

Ovaskainen O, Sato K, Bascompte J, Hanski I. 2002. Metapopulation models for extinction threshold in spatially correlated landscapes. *J Theor Biol* 215:95–108.

Pettan-Brewer KCB, Drew ML, Ramsay E, Mohr FC, Lowenstine LJ. 1996. Herpesvirus particles associat-

ed with oral and respiratory lesions in a California desert tortoise (*Gopherus agassizii*). *J Wildl Dis* 32: 521–526.

Rideout B. 2015. *Transmissible infections and desert tortoise translocation: A comprehensive disease risk analysis*. Desert Tortoise Recovery Office, US Fish and Wildlife Service, Reno, Nevada. https://www.fws.gov/nevada/desert_tortoise/documents/misc/Final_2015desert_tortoise_disease_risk_assessment.pdf. Accessed September 2019.

Rothman KJ, Greenland S, Lash TL. 2008. Chapter 11: Design strategies to improve study accuracy. In: *Modern epidemiology*, 3rd Ed., Rothman KJ, Greenland S, editors. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, pp. 168–182.

Sandmeier FC, Maloney KN, Tracy CR, Hyde D, Mohammadpour H, Marlow R, DuPré S, Hunter K. 2017. Chronic disease in the Mojave Desert tortoise: Host physiology and recrudescence obscure patterns of pathogen transmission. *Ecol Evol* 7:10616–10629.

Scott PA, Allison LJ, Field KJ, Averill-Murray RC, Shaffer HB. 2020. Individual heterozygosity predicts translocation success in threatened desert tortoises. *Science* 370:1086–1089.

Soulé ME. 1980. *Conservation biology: An evolutionary-ecological perspective*. Sinauer Associates, Sunderland, Massachusetts, 432 pp.

USFWS (US Fish & Wildlife Service). 1994. *Desert tortoise (Mojave population) recovery plan*. USFWS, Portland, Oregon, 73 pp.

USFWS. 2011. *Revised recovery plan for the Mojave population of the desert tortoise*. Pacific Southwest Region, Sacramento, California, 246 pp.

USFWS. 2015. *Health assessment procedures for the desert tortoise* (*Gopherus agassizii*): *A handbook pertinent to translocation*. Desert Tortoise Recovery Office, US Fish and Wildlife Service, Reno, Nevada, 75 pp.

USFWS. 2016. *Health assessment procedures for the desert tortoise (Gopherus agassizii): A handbook pertinent to translocation*. Desert Tortoise Recovery Office, US Fish and Wildlife Service, Reno, Nevada, 75 pp.

Vamstad M, Lalumiere K, Root S. 2013. *2012 Annual report for the monitoring program to assess the effects of curbing on desert tortoise movement and survival, JOTR*. Joshua Tree National Park, National Park Service, Twentynine Palms, California, 65 pp.

Woodroffe R, Donnelly CA, Cox DR, Gilks P, Jenkins HE, Johnston WT, Le Fevre AM, Bourne FJ, Cheeseman CL, Clifton-Hadley RS, et al. 2009. Bovine tuberculosis in cattle and badgers in localized culling areas. *J Wildl Dis* 45:128–143.

Weitzman CL, Sandmeier FC, Tracy CR. 2017. Prevalence and diversity of the upper respiratory pathogen *Mycoplasma agassizii* in Mojave desert tortoises (*Gopherus agassizii*). *Herpetologica* 73:113–120.

Wendland LD, Zacher LA, Klein PA, Brown DR, Demcovitz D, Littell R, Brown MB. 2007. Improved enzyme-linked immunosorbent assay to reveal *Mycoplasma agassizii* exposure: A valuable tool in the management of environmentally sensitive tortoise populations. *Clin Vaccine Immunol* 14:1190–1195.

Submitted for publication 25 July 2020.
Accepted 4 February 2021.