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Mycoplasmosis in Free-ranging Desert Tortoises in Utah and Arizona

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ABSTRACT: Upper respiratory tract disease (URTD) has been associated with major losses of free-ranging desert tortoises (*Gopherus agassizii*) in the southwestern United States. This prompted a clinical examination of 63 free-ranging desert tortoises for signs of URTD and sampling for *Mycoplasma agassizii,* the causative agent of URTD. Tortoises were sampled from three sites in the eastern Mojave Desert (1992–93), and from three sites in the Sonoran Desert (1992–94). Plasma samples were tested for antibodies to *M. agassizii* using an enzyme-linked immunosorbent assay (ELISA). Nasal aspirate samples from 12 Sonoran tortoises were tested using polymerase chain reaction (PCR) test directed at the 16S rRNA gene of *M. agassizii.* Nasal aspirate samples from all tortoises were cultured for *M. agassizii.* In the Mojave Desert, nine tortoises had clinical signs of URTD and eight were seropositive for *M. agassizii.* In the Sonoran Desert, there were no clinical signs of URTD, but two tortoises were seropositive, and two tortoises had positive PCR results.
Key words: Arizona, desert tortoise, *Gopherus agassizii,* Mojave Desert, *Mycoplasma agassizii,* Sonoran Desert, upper respiratory tract disease, Utah.

Population declines of the desert tortoise (*Gopherus agassizii*) led to listing of the Mojave desert tortoise as threatened on 2 April 1990 (US Fish and Wildlife Service, 1990). This population of desert tortoises is located north and west of the Colorado River (USA), and mortality caused by upper respiratory tract disease (URTD) was considered as a contributor to its rapid decline (Corn, 1994). The Sonoran tortoise population (located south and east of the Colorado River) was a candidate for listing in 1991, but the US Fish and Wildlife Service determined that listing was not warranted because this population seemed stable and healthy (US Fish and Wildlife Service, 1991). Prompted by increasing concerns about the impact of URTD on tortoise populations, the Arizona Game and Fish Department initiated two 5-yr health studies; the first of Mojave tortoises in northern Arizona and southern Utah (1989–1993), and the second of Sonoran tortoises in central Arizona (1990–1994). The objectives of both studies were to determine the extent of URTD in these populations, and to test for *Mycoplasma agassizii,* the causative agent of URTD (Brown et al., 1994).

Tortoises were sampled at three study sites in the northeastern Mojave Desert: City Creek in Washington County, Utah (37°10′N, 113°35′W) (n=13); Paradise Canyon in Washington County, Utah (37°9′N, 113°36′W) (n=1); and Littlefield in Mohave County, Arizona (37°4′N, 113°55′W) (n=14). Tortoises were sampled at three study sites in the central Sonoran Desert: Little Shipp Wash in Yavapai County, Arizona (34°31′N, 113°5′W) (n=12); Harcuvar Mountains, La Paz County, Arizona (34°6′N, 113°17′W) (n=11); and Sand Tank Mountains on the Barry Goldwater Bombing Range, Maricopa County, Arizona (32°37′N, 112°22′) (n=12). Vegetation at the Mojave sites was Mojave desertscrub (Turner, 1994), and the vegetation at the Sonoran sites was upland Sonoran desertscrub (Turner and Brown, 1994). Rainfall in the upland Sonoran desertscrub occurs in both winter and late summer, while Mojave desertscrub receives only winter rains (Turner, 1994).

All desert tortoises included in this

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study were free-ranging adults (>208 mm median carapace length [MCL]). They were radio tagged (Dickinson et al., 2002), recaptured, and sampled three times a year (May, July, September) at City Creek and Littlefield (1992–93), and at Little Shipp and the Harcuvars (1993–94). The tortoise captured at Paradise Canyon was sampled twice in 1992 and three times in 1993. Tortoises in the Sand Tank Mountains were sampled once (1992). Some tortoises were not sampled in every period because of capture difficulties or lost signals.

At each capture, tortoises were individually placed in a clean pillow case and transported to a field laboratory where they were physically examined for evidence of URTD, weighed and measured as described in Dickinson et al. (2002), and blood and nasal aspirate were collected. Evidence of URTD was indicated by any nasal or ocular mucous discharge, occluded nares, conjunctivitis, or palpebral edema. Tortoises were handled individually with new gloves and kept in clean, individual cardboard boxes to minimize the possibility of disease transfer among animals.

Tortoises were immobilized within 4–6 hr after capture for blood collection as described (Dickinson et al., 2002). Twenty minutes after immobilization, 1.0 ml of whole blood was collected by jugular venipuncture (Jacobson et al., 1992) and placed in a lithium heparin microtainer (Becton Dickinson, Rutherford, New Jersey, USA). After mixing for 5 min, plasma (approximately 0.5 ml) was separated by centrifugation, placed in a cryogenic vial (Whatman LabSales, Hillsboro, Oregon, USA), and immediately frozen in liquid nitrogen. Plasma samples were mailed on dry ice to the Immunological Analysis Laboratory, Interdisciplinary Center for Biotechnology Research, University of Florida (Gainesville, Florida, USA) within 2 days of collection. Plasma was tested for the presence of *M. agassizii* antibodies using an enzyme-linked immunosorbent assay (ELISA) (Schumacher et al., 1993).

For each tortoise, nares were flushed with an open-end 3.5-ml catheter (Sherwood Medical, St. Louis, Missouri, USA) attached to a 3.0-ml syringe filled with 0.25 ml of 0.9% sodium chloride (Abbott Laboratories, Chicago, Illinois, USA). The aspirate from both nares was placed into a single cryogenic vial containing 0.5 ml of tryptic soy broth (MicroBio Products, Tempe, Arizona, USA). The sample was mixed, and immediately frozen in liquid nitrogen.

In 1994, Sonoran tortoise nasal aspirates from Little Shipp ($n=6$) and Harcuvars ($n=6$) were tested for *M. agassizii* by polymerase chain reaction (PCR) at the Department of Pathobiology, College of Veterinary Medicine, University of Florida. The PCR test was designed to detect the 16S ribosomal ribonucleic acid (rRNA) gene of mycoplasmas (Brown et al., 1995). Tortoises were rehydrated and released after sampling as described in Dickinson et al. (2002).

ELISA results were reported as an enzyme immunosorbent assay (EIA) ratio (EIA ratio=A_{405} of sample/A_{405} of negative control, where A_{405}=spectrophotometer absorbance at 405 nm). Enzyme immunosorbent assay ratios ≥2 were interpreted as suspect, and ratios ≥3 were considered antibody positive.

Twenty-eight Mojave tortoises (19 males, nine females) and 35 Sonoran tortoises (21 males, 14 females) were sampled. In 5 yr of study, only nine (14%) tortoises had clinical signs of URTD. Eight of nine tortoises with URTD were from the Littlefield site, and one tortoise was from Paradise Canyon.

Nine tortoises from the Mohave Desert sites tested seropositive for *M. agassizii*. Two tortoises were seropositive in four consecutive sampling periods (September 1992, May 1993, July 1993, September 1993), which coincided with observed URTD. Another tortoise was seropositive three of four sampling periods (September

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1992, May 1993, September 1993), but showed no clinical signs of URTD. Only one tortoise seemed to recover from URTD as it went from seropositive (September 1992; with clinical signs), to suspect (May 1993; with clinical signs), and finally seronegative (July 1993; without clinical signs). The ELISA result for one tortoise was suspect in September 1992 and then was seronegative for subsequent recaptures (May 1993, July 1993).

Clinical signs of URTD were not observed in Sonoran tortoises. Antibodies to *M. agassizii* were detected in two animals. Three additional animals were classified as suspect. Two of 12 Sonoran tortoises sampled in 1994 tested positive by PCR, but only one of these PCR-positive animals tested seropositive by ELISA. This inconsistency may reflect a delay in the detectable immune response that typically takes at least 6 wk to develop (Brown et al., 2002).

Of the 68 blood samples from the Mohave Desert, 26% were seropositive. This prevalence is higher than that reported for Mojave tortoises sampled at Yucca Mountain (Nevada, USA) where 19% (45 of 283) of blood samples tested seropositive (Lederle et al., 1997). Lederle et al. (1997) also observed clinical signs of URTD in Mojave tortoises less frequently than reported in this study (0.5% vs. 32%). In another Nevada study, Schumacher et al. (1997) found that 31% (45 of 144) of Mojave tortoises had clinical signs. Schumacher et al. (1997) also reported a correlation between clinical signs and positive serologic results (84%, 38 of 45). Our study showed a similar correlation between clinical signs and positive serologic results (89%; 8 of 9).

Compared with Mojave tortoises, Sonoran tortoises had no evidence of URTD. Of 101 blood samples collected from Sonoran tortoises, <2% were seropositive. Two tortoises tested positive for the *M. agassizii* 16S rRNA gene by PCR. This is the first reported ELISA and PCR data suggesting that *M. agassizii* is present in Sonoran tortoises, but these results were not confirmed by sequencing of PCR products or culture. The reasons for the absence of clinical signs of URTD and the low prevalence of antibodies in the surveyed Sonoran tortoise populations are unknown.

Captive tortoises may be a source of *M. agassizii* to free-ranging populations (Jacobson et al., 1991; Jacobson, 1994), and this could explain the high prevalence of *M. agassizii* in tortoises at our Mojave sites. Captive tortoise releases were reported in the Red Cliffs Desert Reserve (City Creek) and on the Beaver Dam Slope (Littlefield) in the 1970s and 1980s (Duck, unpubl. data). In contrast, the Sonoran sites in this study are located in comparatively remote areas and therefore captive release may be less common.

The average duration of URTD in free-ranging tortoises is unknown. In this study, two Mojave tortoises had clinical signs of URTD for 2 of 5 yr. Jacobson et al. (1991) reported that URTD in free-ranging tortoises was chronic in nature and lasted for as long as 1 yr before death. He further reported that affected captive tortoises "may survive for several years before succumbing to systemic disease." More long-term monitoring of tortoises at the Mojave sites is needed to determine both the duration of URTD and the fate of infected tortoises.

Additional studies to detect *M. agassizzi* in unstudied Sonoran populations are also warranted. Such studies may be especially relevant during times of stress caused by drought, habitat loss, and overcrowding.

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