

Mycoplasmosis in Free-ranging Desert Tortoises in Utah and Arizona

Authors: Dickinson, Vanessa M., Schumacher, Isabella M., Jarchow, James L., Duck, Timothy, and Schwalbe, Cecil R.

Source: Journal of Wildlife Diseases, 41(1) : 239-243

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-41.1.239>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Mycoplasmosis in Free-ranging Desert Tortoises in Utah and Arizona

Vanessa M. Dickinson,^{1,6,7} Isabella M. Schumacher,² James L. Jarchow,³ Timothy Duck,⁴ and Cecil R. Schwalbe⁵ ¹ Research Branch, Arizona Game and Fish Department, 2221 W. Greenway Rd., Phoenix, Arizona, USA; ² 8525 Richland Colony Rd., Knoxville, Tennessee 37923, USA; ³ Sonora Animal Hospital, 410 W. Simons, Tucson, Arizona 85705, USA; ⁴ Bureau of Land Management, Arizona Strip District, 225 N. Bluff, St. George, Utah, 84770, USA; ⁵ National Biological Service, BioSciences East, University of Arizona, Tucson, Arizona 85721, USA; ⁶ Current address: 515 E. Lavitt Lane, Phoenix, Arizona 85086, USA; and ⁷ Corresponding author (email: mcaracara@yahoo.com).

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 68 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 was analyzed for antibodies to *M. agassizii* using an enzyme-linked immunosorbent assay (ELISA). Nasal aspirate from 12 Sonoran tortoises was tested using polymerase chain reaction (PCR) test to detect the 16S rRNA gene of *M. agassizii*. Nasal aspirate from all tortoises was cultured for *Mycoplasma* sp. In the Mojave Desert, nine tortoises had clinical signs of URTD ($n=28$). Eight of the nine tortoises with clinical signs were seropositive for *M. agassizii*. In the Sonoran Desert, there were no clinical signs of URTD, but two tortoises were seropositive ($n=40$), and two tortoises had PCR results indicating presence of *M. agassizii* ($n=12$). Monitoring of URTD is recommended for Mojave tortoises, and further monitoring is needed for Sonoran tortoises because we do not know the extent of this disease in this population.

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 the Mojave desert tortoise as threatened in 1990 (U.S. Fish and Wildlife Service, 1990). The listing prompted studies of diseases of Mojave tortoises; a population of desert tortoises located north and west of the Colorado River (USA). Mortality caused by upper respiratory tract disease (URTD) was considered a factor in the rapid decline in the Mojave population

(Corn, 1994). The Sonoran tortoise population (located south and east of the Colorado River) was a candidate for listing in 1991, but the U.S. Fish and Wildlife Service found that listing was not warranted because this population seemed stable and healthy (U.S. 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 (1989–93); and the second of Sonoran tortoises in central Arizona (1990–94). The objectives of both studies were to determine the extent of URTD in Mojave and Sonoran populations and to test for *M. agassizii*, the causative agent of URTD (Brown et al., 1994) in both populations.

Three study sites were selected in the northeastern Mojave Desert: City Creek in Washington County, Utah, USA (37°10'N, 113°35'W); Paradise Canyon in Washington County, Utah, USA (37°9'N, 113°36'W); and Littlefield in Mohave County, Arizona, USA (37°4'N, 113°55'W). Three study sites were selected in the central Sonoran Desert: Little Shipp Wash in Yavapai County, Arizona, USA (34°31'N, 113°5'W); Harcuvar Mountains, La Paz County, Arizona, USA (34°6'N, 113°17'W); and Sand Tank Mountains on the Barry Goldwater Bombing Range, Maricopa County, Arizona, USA (32°37'N, 112°22'). Vegetation at the Mojave sites was Mojave desertscrub (Turner, 1994), and the vegetation at the Sonoran sites was upland Son-

21

oran desertscrub (Turner and Brown, 1994). Rainfall in the upland Sonoran desertscrub occurs in both winter and late summer whereas Mojave desertscrub receives only winter rains (Turner, 1994).

All desert tortoises in this study were free-ranging adult (>208-mm median carapace length, MCL). They were radio-tagged (Dickinson et al., 2002), subsequently 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). Only one tortoise was captured and sampled at Paradise Canyon (sampled twice in 1992 and three times in 1993). Tortoises in the Sand Tank Mountains were sampled once (1992).

At each capture, tortoises were transported to a field laboratory individually in clean pillow cases. At the field laboratory, tortoises were physically examined for evidence of URTD, weighed and measured as described in Dickinson et al. (2002), and sampled for blood and nasal aspirate. 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 of capture for blood collection as described in Dickinson et al. (2002). Twenty minutes after immobilization, 1.0 ml of whole blood was collected by jugular venipuncture (Jacobson et al., 1992). Whole blood was placed in a lithium heparin microtainer (Becton Dickinson, Rutherford, New Jersey, USA), mixed for 5 min, and then centrifuged for 5 min. Plasma (0.5 ml) was pipetted off, placed in cryogenic vials (Whatman LabSales, Hillsboro, Oregon, USA), and immediately frozen in liquid nitrogen. Plasma samples were placed on dry ice and mailed to the Immunological Analysis Laboratory, Interdisciplinary Center for Biotechnology Re-

search, University of Florida (Gainesville, Florida, USA) within 2 days of collection. An aliquot was tested for the presence of *M. agassizii* antibodies using an enzyme-linked immunosorbent assay (ELISA) (Schumacher et al., 1993).

Each tortoise naris was flushed with an open-end 3.5-ml catheter (Sherwood Medical, St. Louis, Missouri, USA) attached to a 3-ml syringe filled with 0.25 ml 0.9% sodium chloride (Abbott Laboratories, Chicago, Illinois, USA). The aspirate from both nares was placed into a cryogenic vial containing 0.5 ml of tryptic soy broth (MicroBio Products, Tempe, Arizona), mixed, and immediately frozen in liquid nitrogen.

In 1994, Sonoran tortoise nasal aspirate from Little Shipp and Harcuvars was tested for *M. agassizii* using a polymerase chain reaction (PCR) test at the Department of Pathobiology, College of Veterinary Medicine, University of Florida. The PCR test detected 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).

Results of the ELISA were reported as an enzyme immunoassay (EIA) ratio (EIA ratio = A_{405} of sample : A_{405} of negative control, where A_{405} is the spectrophotometer absorbance at 405 nm). Enzyme immunoassay ratios >2 indicated suspect, and ratios >3 were considered positive. Tortoises were not sampled in every period because of capture difficulties or lost signals. For the normally distributed Sonoran data, differences in EIA ratios between seasons were tested for each sampling period by desert using one-way analysis of variance (ANOVA). The Mojave data were not normally distributed (Lilliefors' test; Lilliefors, 1967), and nonparametric statistics were used. Kruskal-Wallis ANOVA were used to test differences between seasons for each sampling period.

Sixty-eight tortoises were examined and sampled: 28 Mojave tortoises (19 males,

TABLE 1. Results of enzyme-linked immunosorbent assay for *Mycoplasma agassizii* antibodies on blood samples from desert tortoises from the Mojave Desert and the Sonoran Desert.

Site and period of sample collection	EIA ^a results				
	<i>N</i>	<i>X</i>	SE	<i>H</i> ^b	<i>P</i>
Mojave Desert					
September 1992	21	7.6	16.9	2.42	0.12
May 1993	20	5.3	11.4	0.51	0.47
July 1993	12	9.6	17.1	1.44	0.23
September 1993	15	7.6	13.1	0.025	0.87
Sonoran Desert					
September 1992	19	0.9	0.5	3.62	0.07
May 1993	14	1.1	0.3	0.003	0.95
July 1993	13	1.4	0.4	0.76	0.40
September 1993	16	0.6	0.2	0.11	0.74
May 1994	11	0.7	0.1	1.19	0.30
July 1994	12	0.8	0.2	2.17	0.17
September 1994	16	1.2	0.8	0.76	0.39

^a EIA = enzyme immunosorbent assay ratio; *N* = number of tortoises tested; *X* = mean untransformed EIA ratios.

^b *H* = Kruskal-Wallis analysis of variance for non-normal data with probability (*P*).

^{ca} *F* = one-way analysis of variance with probability (*P*).

nine females) and 40 Sonoran tortoises (24 males, 16 females). In 5 years of study, only nine (13%) tortoises had clinical signs of UR TD. All tortoises with clinical signs of UR TD were from the Mojave population, with the Littlefield site having the most clinically ill animals (67%; *n*=9). Two tortoises (Littlefield 213, Paradise Canyon 12) were observed for 2 yr with clinical signs of UR TD in every season. Littlefield tortoise H036 was observed for 5 yr. During the first 4 yr, this tortoise had no clinical signs, but in the fifth year (1993) it had clinical signs in each sampling period, lost weight in each subsequent season, and was dehydrated in the last sampling period.

Blood sample sizes, mean EIA ratio, and statistical results for all sampled Mojave and Sonoran populations are listed on Table 1. The likelihood of testing positive for *M. agassizii* antibodies for Mojave and Sonoran tortoises did not differ between seasons ($P > 0.05$; Table 1). Of the nine Mojave tortoises with clinical signs of UR TD, eight tortoises were seropositive for *M. agassizii* for at least one season, and one tortoise was suspect (*n*=28). Two tortoises (Littlefield 213, Paradise Canyon 12) were seropositive in four consecutive

sampling periods (September 1992, May 1993, July 1993, September 1993), which coincided with clinical signs of UR TD. All tortoises with seropositive titers for *M. agassizii* showed clinical signs of UR TD, except one. Littlefield tortoise 250 was seropositive three of four sampling periods (September 1992, May 1993, September 1993), but had no clinical signs of UR TD. Only one tortoise (City Creek 1144) seemed to recover from UR TD as it went from seropositive (September 1992; clinical signs), to suspect (May 1993; clinical signs), and finally seronegative (July 1993; no clinical signs). One Mojave tortoise had a suspect antibody titer (City Creek 1126) in September 1992 and then was seronegative for subsequent recaptures (May 1993, July 1993).

There were no clinical signs of UR TD in Sonoran tortoises, but seropositive and suspect titers were found, as possible evidence of the *M. agassizii* 16S rRNA gene. Two Sonoran tortoises were seropositive (Harcuvar 208, Sand Tanks 21), and three were suspect (*n*=40). Harcuvar tortoise 208 was seropositive in its last sampling period of seven sampling periods. The PCR test conducted on 12 Sonoran tor-

toises in 1994 resulted in two weak positive results (Little Shipp 500, Harcuvar 208). Harcuvar 208 may have had URTD because it also tested seropositive with ELISA, but Little Shipp 500 tested seronegative with ELISA. This may indicate a recent infection of Little Shipp 500 by *M. agassizii* because the ELISA depends on an immune response that typically takes at least 6 wk to develop (Brown et al., 2002).

Of the 68 blood samples collected from tortoises from the Mojave, 26% were seropositive ($n=28$). This value is higher than that in Mojave tortoises sampled at Yucca Mountain (Nevada, USA) where out of 283 blood samples, only 19% were seropositive (Lederle et al., 1997). Lederle et al. (1997) also observed clinical signs of URTD in Mojave tortoises less frequently than this study (0.5% compared with 32%). In another Nevada study, Schumacher et al. (1997) found that 31% (45/144) of Mojave tortoises had clinical signs. Schumacher et al. (1997) also reported a correlation between clinical signs and positive test results (84%, 38/45). Our study also showed a similar correlation between clinical signs and positive test results (89%; 8/9).

Compared with Mojave tortoises the Sonoran tortoises had less evidence of URTD. Of 101 Sonoran tortoise blood samples collected, <2% were seropositive with no clinical signs of URTD found ($n=40$). Two tortoises tested positive for the *M. agassizii* 16S rRNA gene ($n=12$). This is the first reported ELISA and PCR data for URTD in Sonoran tortoises. The reasons for the absence of clinical signs of URTD, few seropositive animals, and little evidence of the presence of *M. agassizii* by PCR in Sonoran tortoises are still unknown.

Captive tortoises may be a source of *M. agassizii* and can consequently infect free-ranging populations (Jacobson et al., 1991; Jacobson, 1994). This could be a factor in the high prevalence of *M. agassizii* in tortoises at our Mojave sites. There have been reports of captive tortoise releases

within the Red Cliffs Desert Reserve (City Creek) and on the Beaver Dam Slope (Littlefield) in the 1970s and 1980s (T. Duck, unpubl. data). In contrast, the Sonoran sites in this study are located in comparatively remote areas, and therefore, release of captive tortoises may be less common.

The average duration of URTD in free-ranging tortoises is debatable. In this study, two Mojave tortoises had clinical signs of URTD in 2 of 5 yr. Jacobson et al. (1991) reported URTD in free-ranging tortoises was chronic and that clinical signs lasted for as long as 1 yr before death. He reported ill tortoises in captivity "may survive for several years before succumbing to systemic disease." More long-term monitoring of tortoises at the Mojave site may find tortoises living longer with URTD than expected and that recovery is more common than previously thought. Additional studies of the prevalence of *M. agassizii* in the relatively unstudied Sonoran population is also warranted. Monitoring individual tortoises is recommended in sites with small, isolated populations, especially during times of stress caused by drought, habitat loss, and overcrowding.

We thank assistants J. Snider, S. Trachy, and D. Parmley, and interns L. Sychowski, A. Carnahan, J. Hurst, P. Lowe, and many federal, state, and university volunteers for data collection. M. Christopher and J. deVos provided constructive comments. Financial support for this project was provided by the Bureau of Land Management, Arizona Game and Fish Department Heritage Fund, U.S. Fish and Wildlife Service, and the Utah Division of Wildlife Resources.

LITERATURE CITED

- BROWN, M. B., I. M. SCHUMACHER, P. A. KLEIN, K. HARRIS, T. CORRELL, AND E. R. JACOBSON. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infection and Immunity* 1994: 4580–4586.
- BROWN, D. R., B. C. CRENSHAW, G. S. MCLAUGHLIN, I. M. SCHUMACHER, C. E. MCKENNA, P. A. KLEIN, E. R. JACOBSON, AND M. B. BROWN. 1995. Taxonomic analysis of the tortoise myco-

- plasmas *Mycoplasma agassizii* and *Mycoplasma testudinis* by 16S rRNA gene sequence comparison. *International Journal of Systematic Bacteriology* 45: 348–350.
- , I. M. SCHUMACHER, G. S. MCLAUGHLIN, L. D. WENDLAND, M. B. BROWN, P. A. KLEIN, AND E. R. JACOBSON. 2002. Application of diagnostic tests for mycoplasmal infections of desert and gopher tortoises, with management recommendations. *Chelonian Conservation and Biology* 4: 497–507.
- CORN, P. S. 1994. Recent trends of desert tortoise populations in the Mojave Desert. *In* *Biology of North American tortoises*, R. B. Bury and D. J. Germano (eds.). U.S. National Biological Survey, Fish and Wild Research Report 13. U.S. Fish and Wildlife Service, Washington, D.C., pp. 85–93.
- DICKINSON, V. M., J. L. JARCHOW, AND M. H. TRUEBLOOD. 2002. Hematology and plasma chemistry reference ranges for free-ranging desert tortoises in Arizona. *Journal of Wildlife Diseases* 38: 143–153.
- JACOBSON, E. R. 1994. Causes of mortality and disease in tortoises: A review. *Journal of Zoo and Wildlife Medicine* 25: 2–17.
- , J. M. GASKIN, M. B. BROWN, R. K. HARRIS, C. H. GARDINER, J. L. LAPOINTE, H. P. ADAMS, AND C. REGGIARDO. 1991. Chronic upper respiratory disease of free-ranging desert tortoises (*Xerobates agassizii*). *Journal of Wildlife Diseases* 27: 296–316.
- , J. SCHUMACHER, AND M. GREEN. 1992. Field and clinical techniques for sampling and handling blood for hematologic and selected biochemical determinations in the desert tortoise *Xerobates agassizii*. *Copeia* 1992: 237–241.
- LEDERLE, P. E., K. R. RAUTENSTRAUCH, D. L. RAKESTRAW, K. K. ZANDER, AND J. L. BOONE. 1997. Upper respiratory tract disease and mycoplasmosis in desert tortoises from Nevada. *Journal of Wildlife Diseases* 33: 759–765.
- LILLIEFORS, H. W. 1967. On the Kolmogorov-Smirnor test for normality with mean and variance unknown. *Journal of the American Statistical Association* 64: 399–402.
- SCHUMACHER, I. M., M. B. BROWN, E. R. JACOBSON, B. R. COLLINS, AND P. A. KLEIN. 1993. Detection of antibodies to a pathogenic mycoplasma in desert tortoises (*Gopherus agassizii*) with upper respiratory tract disease. *Journal of Clinical Microbiology* 1993: 1454–1460.
- , D. B. HARDENBROOK, M. B. BROWN, E. R. JACOBSON, AND P. A. KLEIN. 1997. Relationship between clinical signs of upper respiratory tract disease and antibodies to *Mycoplasma agassizii* in desert tortoises from Las Vegas Valley, Nevada. *Journal of Wildlife Diseases* 33: 261–266.
- STATSOFT, INC. 1994. *Statistica*, Vol. 1. General statistics I. StatSoft Inc., Tulsa, Oklahoma, pp. 1455–1456.
- TURNER, R. M. 1994. Mojave desertscrub. *In* *Biotic communities of the American southwest*, D. E. Brown, (ed.). University of Arizona, Tucson, Arizona, pp. 157–168.
- , AND D. E. BROWN. 1994. Sonoran Desertscrub. *In* *Biotic communities of the American southwest*, D. E. Brown, (ed.). University of Arizona, Tucson, Arizona, pp. 181–221.
- U.S. FISH AND WILDLIFE SERVICE. 1990. Endangered and threatened wildlife and plants; determination of threatened status of the Mojave population of the desert tortoise. *Federal Register* 55: 12178–12191.
- . 1991. Endangered and threatened wildlife and plants; findings on a petition to list the Sonoran desert tortoise as threatened or endangered. *Federal Register* 56:29453–29455.

Received for publication 22 August 2003.