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NASAL AND CLOACAL BACTERIA IN FREE-RANGING DESERT TORTOISES FROM THE WESTERN UNITED STATES

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ABSTRACT: Aerobic bacteria were collected from three free-ranging desert tortoise (*Gopherus agassizii*) populations in the eastern Mojave Desert (Arizona, Utah; USA) from 1989 to 1993, and from two free-ranging populations in the central Sonoran Desert (Arizona, USA) from 1990 to 1994. Six species of nasal bacteria and 18 species of cloacal bacteria were identified. At least one potential pathogen was found in the nasal cavity (*Pasteurella testudinis*), and at least two potential pathogens in the cloaca (*Pseudomonas* spp., *Salmonella* spp.).

Key words: Bacteria, desert tortoise, *Gopherus agassizii,* Mojave Desert, *Pasteurella testudinis, Pseudomonas* spp., *Salmonella* spp., survey.

INTRODUCTION

Concern about population declines in the Mojave desert tortoise (*Gopherus agassizii*) led to a threatened listing on 2 April 1990 (U.S. Fish and Wildlife Service, 1990). This listing was prompted by discovery of upper respiratory tract disease (URTD) in Kern County (California, USA; Jacobson et al., 1991). The disease spread rapidly throughout the tortoise population and resulted in drastic declines. The causative agent of URTD was debated; was it a bacterium, virus, or mycoplasma? The incidence of bacteria in desert tortoises throughout their range was thus important information. The listing prompted two 5-yr health studies of free-ranging tortoises, one in the northeastern Mojave Desert (USA) and one in the central Sonoran Desert (USA). Few studies have reported on desert tortoise bacteria (Fowler, 1977; Snipes and Biberstein, 1982; Jackson and Needham, 1983; Jacobson et al., 1991). Our objectives were to collect baseline data on aerobic nasal and cloacal bacteria found on nasal and cloacal epithelium of free-ranging desert tortoises; determine if differences occurred as a result of site, sex, season; and determine if we could differentiate between ill and healthy tortoises based on bacteria types.

MATERIALS AND METHODS

Three study sites were selected in the northeastern Mojave Desert including City Creek (Washington County, Utah; 37°10′N, 113°35′W), Littlefield (Mohave County, Arizona; 37°4′N, 113°55′W), and Paradise Canyon (Washington County, Utah; 37°9′N, 113°36′W). The two selected sites in the central Sonoran Desert were Little Shipp Wash (Yavapai County, Arizona; 34°31′N, 113°5′W) and the Harcuvar Mountains (La Paz County, Arizona; 34°6′N, 113°17′W). Vegetation at the Mojave sites was Mojave desert scrub, and vegetation at the Sonoran sites was Sonoran upland scrub (Turner and Brown, 1984).

A maximum of 20 adult (>208 mm median carapace length [MCL]) free-ranging desert tortoises were sampled at both City Creek and Littlefield, and a maximum of 15 tortoises were sampled at both Little Shipp Wash and the Harcuvar Mountains. Only one tortoise was captured and sampled at Paradise Canyon. Most study animals were captured in their shelter sites or out in the open in 1989 in the Mojave Desert, and in 1990 in the Sonoran Desert. We primarily located tortoises by shining a light into underground shelters. Once an adult tortoise was found, 5 min gel expoxy was used to affix radio transmitters (Model 125, Telonics, Mesa, Arizona, USA) to its anterior marginal scutes. We radio-tagged tortoises in order to gather and interpret health data on individual tortoises throughout seasons and years. The transmitters weighed 47 to 53 g, measured 4.1 cm × 2.4 cm × 2.0 cm, and had an active life of 9 to 18 mo. Both male and female tortoises were captured and sampled.

The sex of tortoises was determined by plastron indentation, tail morphology, and gular size (Woodbury and Hardy, 1948). When sex could not be accurately determined, tortoise sex was classified as unknown. A minimum of five and a maximum of 20 adult tortoises were captured during each sampling trip. One sampling trip was made to the Mojave Desert sites in 1989 (September 1989), and then each site was sampled three times a year (May, July, September) from 1990 to 1993. The exception was Paradise Canyon, which was sampled two times in 1992 and three times in 1993. The Paradise Canyon was added to the sampling schedule in July 1992, following discovery of an adult male tortoise with clinical signs of upper respiratory tract disease (URTD). The Sonoran Desert sites were sampled twice in 1990 (September, November), and then sampled each site three times a year (May, July, September) from 1991 to 1994.

At each recapture, tortoises were physically examined for signs of disease, weighed with a 5 kg Pesola scale, and measured with a caliper and 24 cm ruler. Tortoises were considered to have signs of URTD if they showed nasal discharge, conjunctivitis, or wheezing. In this study, ill tortoises refer to tortoises with clinical signs of URTD or positive test results for the causative agent of URTD, *Mycoplasma agassizii* (Brown et al., 1994). We tested tortoise plasma (0.5 ml) with an enzyme-linked immunosorbent assay (ELISA; Schumacher et al., 1993), and tortoise nasal aspirate (0.25 ml nasal flush, stored in 0.5 ml of tryptic soy broth) with a polymerase chain reaction (PCR) test for the 16s rRNA found in *M. agassizii* (Brown and Brown 1994). Mojave tortoises were tested with ELISA (1992–93), and Sonoran tortoises had both ELISA (1992–94) and PCR tests (1994). All tortoises were handled with latex gloves, gloves were changed between tortoises, and tortoises were kept in clean, individual cardboard boxes to minimize the probability of disease transfer among animals.

In 1989, nine Mojave tortoises (City Creek) were swabbed at two sampling sites (external nares, choana) and the swabs placed in three transport media (Culturettes from Becton Dickinson, Cockeysville, Maryland, USA), Stuart transport swabs (Starplex Etokicoke, Ontario, Canada), thioglycolate broth (Microbio Products, Tempe, Arizona, USA) to test the effectiveness of each collection procedure. From 1989 to 1990, one nasal swab (external nares) was taken from each Mojave and Sonoran tortoise and stored in Transtube (Medical Wire Equipment Co., Dover, New Jersey, USA). The cloacal swabs were kept on ice and sent to the University of Arizona Veterinary Diagnostic Laboratory (Tucson, Arizona, USA) within 24 hr.

From 1990 to 1994, one Transtube cloacal swab from each Mojave and Sonoran tortoise was collected. Cloacal swabs were kept on ice and sent to Animal Diagnostic Laboratory within 24 hr. Bacterial cultures were grown using MacConkey agar (gram-negative bacteria), Selenite agar (*Salmonella* spp., *Shigella* spp.), Hektoen agar (*Salmonella* spp., *Shigella* spp.), and *Campylobacter* agar. Bacteria were classified as gram positive or gram negative and, when possible, identified to species.

For 4 yr in the Mojave Desert (1990–93) and 5 yr in the Sonoran Desert (1991–94), each tortoise naris was flushed with one open-end 3.5 tom cat catheter (Sherwood Medical from St. Louis, Missouri, USA) attached to a 3 ml syringe filled with 0.9% sodium chloride (Abbott Lab, Chicago, Illinois, USA). The aspirate from both nares was placed in a cryogenic vial containing tryptic soy broth (MicroBio Products, Tempe, Arizona, USA), mixed, and then immediately froze in liquid nitrogen. From 1990 to 1991, 0.5 ml of sodium chloride was used to flush each naris, and 1.0 ml of tryptic soy broth was used for culturing the aspirate. In 1992, the amount of sodium chloride (0.25 ml) and tryptic soy broth (0.5 ml) was reduced to replicate procedures used on Mojave desert tortoises in California, USA.

Bacteria were recorded as present or absent. We calculated percentages of bacteria as representation of incidence. A media, Kruskal-Wallis Analysis of Variance (ANOVA; Statistica©, Statsoft, 1994) was used to evaluate differences among cloacal bacteria on complete data sets (1992, 1993). The Kruskal-Wallis ANOVA is a nonparametric alternative to between-groups one-way ANOVA. The test is basically identical to a parametric one-way ANOVA, except that it is based on ranks rather than means (Statsoft, 1994). The median test is a crude version of the Kruskal-Wallis ANOVA as it calculates the number of cases in each sample that falls above or below the common median and computes the *Chi-Square* (χ^2). Significance of the ANOVA was judged at $P < 0.05$.

RESULTS

We captured a total of 92 tortoises in the Mojave desert (42 from City Creek, 49 from Littlefield, 1 from Paradise Canyon). Forty-seven (51%) tortoises were not radio-tagged and were only sampled once. The remaining tortoises were captured at least three times. Only four City Creek tortoises were captured and sampled each

TABLE 1. Bacteria isolated from the cloacal cavity and nasal cavity of Mojave[a] and Sonoran desert sites[b] from 1989 to 1993 and 1990 to 1994, respectively. Organisms were collected with nasal flushes unless indicated.

Genera		Mojave tortoise		Sonoran tortoise	
		Number positive	*n*	Number positive	*n*
Bacillus spp.	Cloacal			13	201
Campylobacter spp.	Cloacal	1	5	9	201
Citrobacter amolonaticus	Cloacal	2	77		
Citrobacter spp.	Cloacal	1	77	10	201
Coliforms	Cloacal	3	56	12	201
Corynebacterium spp.	Cloacal	10	138	13[c]	105
	Nasal			2	25
Diptheroids	Cloacal	6	138	39	201
Enterobacter-Klebsiella	Cloacal	75	138	66	201
Escherichia coli	Cloacal	37	138	31	201
Flavobacterium spp.	Nasal	1[b]	10		
Lactobacillus spp.	Cloacal	7	77	18	201
Pasteurella testudinis	Cloacal	12	77		
	Nasal	49	221		
Pasteurella spp.	Cloacal	5	133	7	201
	Nasal			12	26
Proteus spp.	Cloacal			3[d]	105
Pseudomonas spp.	Cloacal	59	138	58	201
	Nasal			5	6
Salmonella spp.	Cloacal	3	212	18	201
Shiegella spp.	Cloacal	25	172	42	201
Staphylococcus spp.	Cloacal	130	138	151	201
	Nasal	5[c]	12	4[d]	20
Streptococcus spp.	Cloacal	15	133	17	201
	Nasal			1	6
Yeast	Cloacal	2	77		

[a] City Creek, Utah, USA; Littlefield, Arizona, USA; Paradise Valley, Utah, USA.
[b] Little Shipp Wash, Arizona, USA; Harcuvar Mountains, Arizona, USA.
[c] Nasal and choanal swabs, and thioglycolate broth (City Creek tortoise).
[d] Harcuvar Mountain tortoise only.

season for 5 yr. Of the remaining 45 tortoises, 12 had clinical signs of URTD. At the end of the Mojave study (1993) 12 ill tortoises (clinical signs of URTD, positive ELISA and PCR) still survived. We lost 17 (18%) tortoise radio signals either from radio transmitter failure or animals leaving the study site. We recorded two mortalities (1 in City Creek, 1 in Littlefield), causes unknown. Neither tortoise showed signs of URTD (found dead prior to 1992 ELISA tests).

We captured 36 tortoises in the Sonoran desert (13 from Little Shipp, 23 from Harcuvars). We recorded 14 mortalities (39%), five of which were attributed to mountain lion (*Felis concolor*) predation. The remaining seven mortalities remains a mystery. None of the captured 36 tortoises had clinical signs of URTD.

In tortoises from the Mojave Desert, three species of bacteria were found in the nasal cavity, with one potential pathogen (*Pasteurella testudinis*) (Table 1). *Pasteurella testudinis* was isolated in 22% (49/221) of the nasal samples. Only one species of bacteria was isolated with swabs (nasal, choana) and thioglycolate broth (*Flavobacterium* spp.). *Staphylococcus* spp. was found five times, with swags (nasal, choana). *Pasteurella testudinis* was isolated with nasal flushes, choanal swabs, and nasal swabs. Compared to healthy tortoises, significantly higher levels of *P. tes-*

tudinis ($\chi^2 = 31.9$, df = 1, $P < 0.001$) were found in ill Mojave desert tortoises and higher levels of *P. testudinis* ($\chi^2 = 7.5$, df = 1, $P < 0.05$) were found in September.

In the Sonoran Desert, five species of bacteria were found in the nasal cavity. Only one potential pathogen, *P. testudinis,* was found in the nasal cavity (1990–94). Higher levels ($\chi^2 = 17.0$, df = 4, $P = 0.002$) of *P. testudinis* were found in 1991 compared to all other years.

In tortoises from the Mojave Desert, two of 17 species of cloacal bacteria were considered opportunistic pathogens (*Pseudomonas* spp., *Salmonella* spp.). Some cloacal bacteria showed site, season, and year differences. The majority (94%) of cloacal bacteria were nonpathogenic *Staphylococcus* spp. (130/138) (Table 3).

In tortoises from the Mojave Desert, three cloacal bacteria showed site differences, four showed seasonal differences, and three showed yearly differences. City Creek tortoises had significantly higher levels of coliforms ($\chi^2 = 4.1$, df = 1, $P < 0.05$) and *Escherichia coli* ($\chi^2 = 4.9$, df = 1, $P < 0.05$) as compared to Littlefield. *Campylobacter* spp. was found only once (Paradise Canyon tortoise # 12; ill). In healthy Mojave tortoises, higher levels of *Corynebacterium* spp. and *Pasteurella* spp. were found in May and higher levels of coliforms and *E. coli* were found in July ($P < 0.05$). Higher levels of *Corynebacterium* spp. ($\chi^2 = 6.8$, df = 1, $P < 0.01$) and *Pasteurella* spp. ($\chi^2 = 4.1$, df = 1, $P < 0.05$) were found in 1992, and *E. coli* ($\chi^2 = 7.5$, df = 1, $P < 0.01$) were found in 1993. There was no difference in numbers of cloacal bacteria by sex for both ill and healthy tortoises.

In tortoises from the Sonoran Desert, 16 species of cloacal bacteria were found (Table 1), two of which were considered opportunistic pathogens (*Pseudomonas* spp., *Salmonella* spp.). The majority (75%) of cloacal bacteria were nonpathogenic *Staphylococcus* spp. (151/201).

One Sonoran tortoise cloacal bacteria showed site differences, three showed seasonal differences, and two showed yearly differences. Little Shipp Wash tortoises had higher levels of *Shigella* spp. ($\chi^2 = 7.8$, df = 1, $P = 0.005$) compared to Harcuvar Mountain tortoises. Higher levels of coliforms ($\chi^2 = 29.0$, df = 2, $P = 0.00$) were found in July, while higher levels of diptheroids ($\chi^2 = 26.0$, df = 2, $P = 0.00$) and *E. coli* ($\chi^2 = 9.3$, df = 2, $P = 0.01$) were found in September compared to all other seasons. Higher levels ($\chi^2 = 31.0$, df = 4, $P = 0.00$) of *Enterobacter-Klebsiella* were found in 1992, and higher ($\chi^2 = 37.9$, df = 4, $P = 0.00$) levels of *Pseudomonas* spp. were found in 1993 compared to all other years. There were no differences ($P > 0.01$) for other cloacal bacteria (*Bacillus* spp., *Campylobacter* spp., *Citrobacter* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Pasteurella* spp., *Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp.) with respect to all other site, season, sex, and year combinations.

DISCUSSION

Ill tortoises had significantly higher levels of *P. testudinis* in their nasal cavities as compared to healthy tortoises. Seven nasal bacteria were isolated, but only one was considered a potential pathogen (*P. testudinis*). Jacobson et al. (1991) found 11 species of bacteria in the respiratory tract (choanae, nasal cavity, trachea, lung) of ill (URTD) and healthy desert tortoises. Most respiratory tract bacteria were found in the choanae; *P. testudinis* was only found in the nasal cavity (Jacobson et al., 1991). Christopher et al. (1997) found high heterophil counts in Mojave desert tortoises with heavy growth of *P. testudinis* in the nasal cavity. Snipes and Biberstein (1982) found *P. testudinis* in ill and healthy captive desert tortoises and reported the "bacterium appears commensal in healthy free-ranging desert tortoises." In this study, ill tortoises had significantly higher numbers of *P. testudinis* in their nasal cavities compared to healthy tortoises.

All tortoises with clinical signs of URTD from 1992–94 had positive ELISA and/or

PCR test results (V. M. Dickinson, unpubl. data). These same tortoises had significantly higher numbers of *P. testudinis* in their nasal cavities. Similarly, Jacobson et al (1995), found greater percentages of *P. testudinis* in affected (ill) tortoises and stated that this bacteria may contribute to the severity of URTD.

In this study, 17 cloacal bacteria were isolated with only two considered pathogens (*Pseudomonas* spp., *Salmonella* spp.). Jacobson (1987) found four bacterial pathogens (*Pseudomonas* spp., *Proteus* spp., *Providencia* spp., *Morganella* spp.) which accounted for 44% of the bacterial isolates, mostly from diseased snakes. *Salmonella* spp. has been frequently cultured from reptile abscesses (Frye, 1981; Marcus, 1981).

In this study, *P. testudinis, Pseudomonas* spp., and *Salmonella* spp. were the least debatable potential pathogens. *Citrobacter* spp., *Corynebacterium* spp., *Enterobacter-Klebsiella, Flavobacterium* spp., *Pasteurella* spp. (other than testudinis), *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., and possibly *Campylobacter* spp. and *Shigella* spp. can, under certain circumstances, be pathogenic to chelonians (Jacobson et al., 1998).

The higher incidence of *P. testudinis*, *Pseudomonas* spp., and *Salmonella* spp. in ill tortoises demonstrates their usefulness in identifying individuals with underlying pathology. In this study these pathogens appear to be truly opportunistic. That is, the replacement of normal, non-pathogenic flora on oronasal and cloacal epithelial surfaces by these potential pathogens was highly indicative of generalized debility and immunosuppression resulting from underlying pathological processes, most notably URTD. Epithelial colonization by these opportunists may lead to further pathology, debilitation, and death if the host in unable to proceed to a recovery phase of the disease.

This study demonstrates the potential for using nasal and cloacal bacteria screens to identify members of the tortoise population that are experiencing underlying pathology, or that are in some way debilitated. This technique is less stressful, less invasive, and the results may be more obvious than hematology/blood chemistry studies. Obviously, this technique of sampling nasal and cloacal bacteria would not identify the cause of debility, but it could be useful as a screen to provide an indication of the overall incidence of debility in a population. Ultimately this technique could allow assessment and comparison of relative health status of different populations of desert tortoises.

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