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Impacts of Upper Respiratory Tract Disease on Olfactory Behavior of the Mojave Desert Tortoise

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ABSTRACT: Upper respiratory tract disease (URTD) caused by *Mycoplasma agassizii* is considered a threat to desert tortoise populations that should be addressed as part of the recovery of the species. Clinical signs can be intermittent and include serous or mucoid nasal discharge and respiratory difficulty when nares are occluded. This nasal congestion may result in a loss of the olfactory sense. Turtles are known to use olfaction to identify food items, predators, and conspecifics; therefore, it is likely that URTD affects not only their physical well-being but also their behavior and ability to perform necessary functions in the wild. To determine more specifically the impact nasal discharge might have on free-ranging tortoises (*Gopherus agassizii*), we compared the responses of tortoises with and without nasal discharge and both positive and negative for *M. agassizii* antibodies to a visually hidden olfactory food stimulus and an empty control. We found that nasal discharge did reduce sense of smell and hence the ability to locate food. Our study also showed that moderate chronic nasal discharge in the absence of other clinical signs did not affect appetite in desert tortoises.

Key words: *Gopherus agassizii*, *Mycoplasma agassizii*, olfaction, scent, URTD.

Turtles, including tortoises, use olfaction for finding food, detecting predators, identifying conspecifics, navigating, and locating mates (Alberts et al. 1994; Graham et al. 1996; Galeotti et al. 2007). The extent to which disease can affect olfaction and subsequent behavior in turtles is not well studied. An epizootic disease affecting the respiratory and olfactory systems was a key factor in the listing of the Mojave population of desert tortoises (*Gopherus agassizii*) as a threatened species under the US Endangered Species Act (US Fish and Wildlife Service 1990; Berry 1997). This upper respiratory tract disease (URTD) is considered a threat to desert tortoise populations and contributing factors should be minimized as part of the recovery of the species (US Fish and Wildlife Service 2011a). Upper respiratory tract disease is associated with several pathogenic agents including *Mycoplasma agassizii, Mycoplasma testudineum, Pasteurella testudinis,* herpesvirus, and an iridovirus (Jacobson 1994; Brown et al. 2004) and coinfections are considered likely. Upper respiratory tract disease caused by *M. agassizii* can be fatal, but most often results in chronic illness manifested as intermittent serous or mucoid nasal discharge (Jacobson et al. 1991). As with other animals, noninfectious etiologies may also cause nasal discharge and could obfuscate diagnoses. An enzyme-linked immunosorbent assay (ELISA) has been used to detect *M. agassizii*–specific antibodies in desert tortoise plasma, and a direct correlation between the presence of nasal mucosal lesions and *M. agassizii*–specific antibodies was identified (Schumacher et al. 1993). This test is used for monitoring *M. agassizii*–induced URTD in free-ranging desert tortoises (Schumacher et al. 1993; US Fish and Wildlife Service 2011b, 2012), and is used as one of the criteria for management actions and decisions regarding desert tortoises. Other methods, including PCR, can be used to detect *M. agassizii*. Although the clinical signs and gross and histologic lesions of *M. agassizii*–induced URTD have been well studied, how this and other chronic infections impact tortoise behavior has not been investigated (Schumacher et al. 1997; Homer et al. 1998; Perez-Heydrich et al. 2012). Nasal discharge associated with

URTD-related pathogens or other causes may result in loss of the olfactory sense, which could have far-reaching consequences including reduced fecundity and survival.

We explored desert tortoise olfactory behavior in relation to presence or absence of clinical signs for URTD. All individuals used for this study were adults (midline carapace length >180 mm). They were split into three groups based on presence or absence of nasal discharge and *M. agassizii* antibody status: 1) no nasal discharge and *M. agassizii*–negative (n=21), 2) no nasal discharge and *M. agassizii*–positive (n=20), and 3) nasal discharge and *M. agassizii*–positive (n=20). Only animals with no observed nasal discharge or those that had moderate to severe serous or mucoid nasal discharge (US Fish and Wildlife Service 2012) were used in this study. All tortoises were bright, alert, responsive, and in good body condition.

Trials were carried out with tortoises (n=61) in outdoor pens at the Desert Tortoise Conservation Center in Las Vegas, Nevada, USA, during April–May 2012. Tortoises held at the Desert Tortoise Conservation Center are fed a supplementary diet of Mazuri Herbivore Diet (PMI Nutrition International, Brentwood, Missouri, USA) in addition to forage growing naturally in the pens. Pens ranged in size from 13.4 m^2 to 58.0 m^2. Air temperature in the shade was 20–35 C during the trials, which is within the tortoise's active temperature range. Ambient air temperatures were taken in full shade 6 cm above the ground prior to each trial. Opaque plastic containers with small holes placed 15 cm from a tortoise's head were used to present scents to the animals without providing a familiar visual stimulus. For each trial, the container held either an olfactory stimulus likely to be of interest to the animals (food; Mazuri Herbivore Diet) or remained empty (blank). Each tortoise underwent two trials, one with a food scent and one blank, alternating the order of the stimulus for each individual. Each trial lasted 5 min and was broken up into 20 15-sec intervals with behavioral observations made from behind a blind where the observer was not visible to the tortoise. Investigative behaviors within a 10-cm radius of the dish were recorded for each interval. These behaviors included sniffing the dish, biting the dish, touching the nose or a limb to the dish, searching adjacent to the dish, and sniffing the adjacent substrate. Each interval that contained one or more investigative behaviors was counted as one positive investigative bout for analysis. Every tortoise was presented with both treatments with a minimum 1-hr rest period between sessions. Following the completion of the second trial, the same food used in the experiment was provided to tortoises with nasal discharge at a similar distance to determine if these individuals were unable to smell the food or were uninterested due to a decreased appetite possibly associated with illness.

Data were analyzed using linear mixed effects models with a Poisson error distribution using packages lme4 and AICcmodavg in R version 2.15.1 (R Development Core Team 2012). Models evaluated to explain the number of investigative bouts included the animal identification number as a random effect to account for repeated measurements, and scent type (food or blank), health status group, and sex of the animal, as well as likely interactions, as fixed factors. Model selection was conducted using Akaike information criterion corrected for small sample size (AICc; Table 1).

The models that best predicted investigative behavior included an interactive effect of the health status group and the scent provided, with some support for a difference due to sex and potential interactions (Table 1). However, examination of the top three models (which comprised 90% of the weight among all models) yielded significant effects due only to the scent provided (blank or food; $P<0.001$ in each case) and the scent by health status group interaction ($P<0.001$ in each case), and a marginally significant effect

TABLE 1. Best fit linear mixed effects models explaining the number of investigative hits performed by desert tortoises (*Gopherus agassizii*) during experimental trials in Nevada, USA. An investigative hit is defined as each 15-sec interval within a trial where a tortoise displayed one or more investigative behaviors toward a dish. Groups included the following: *Mycoplasma agassizii*–negative and no signs of upper respiratory tract disease (URTD), *M. agassizii*–positive and no signs of URTD, and *M. agassizii*–positive and showing signs of URTD. Scent indicates a presence/absence of the food scent stimuli.

Model	K[a]	AICc[a]	ΔAICc	Weight	Cumulative weight
Hits~1+group+scent+scent:group	7	225.91	0	0.38	0.38
Hits~1+sex+group+scent+scent:group	8	226.29	0.38	0.31	0.69
Hits~1+sex+group+scent+scent:sex+scent:group	9	227.26	1.35	0.19	0.89
Hits~1+sex+group+scent+group:sex+scent:group	10	229.2	3.29	0.07	0.96
Hits~1+sex+group+scent+group:sex+scent:sex+scent:group	11	230.36	4.45	0.04	1
Hits~1+group+scent	5	263.84	37.93	0	1
Hits~1+sex+group+scent	6	264.22	38.31	0	1
Hits~1+sex+group+scent+scent:sex	7	265.41	39.5	0	1
Hits~1+sex+group+scent+group:sex	8	267.13	41.22	0	1
Hits~1+scent	3	267.22	41.31	0	1
Hits~1+sex+scent	4	267.8	41.89	0	1
Hits~1+sex+group+scent+group:sex+scent:sex	9	268.32	42.41	0	1
Hits~1+sex+scent+scent:sex	5	268.99	43.08	0	1
Hits~1+group	4	367.77	141.86	0	1
Hits~1+sex+group	5	368.15	142.24	0	1
Hits~1+sex+group+group:sex	7	371.06	145.15	0	1
Hits~1	2	371.15	145.24	0	1
Hits~1+sex	3	371.73	145.82	0	1

[a] K = number of parameters used in the model; AICc = Akaike information criterion corrected for small sample size.

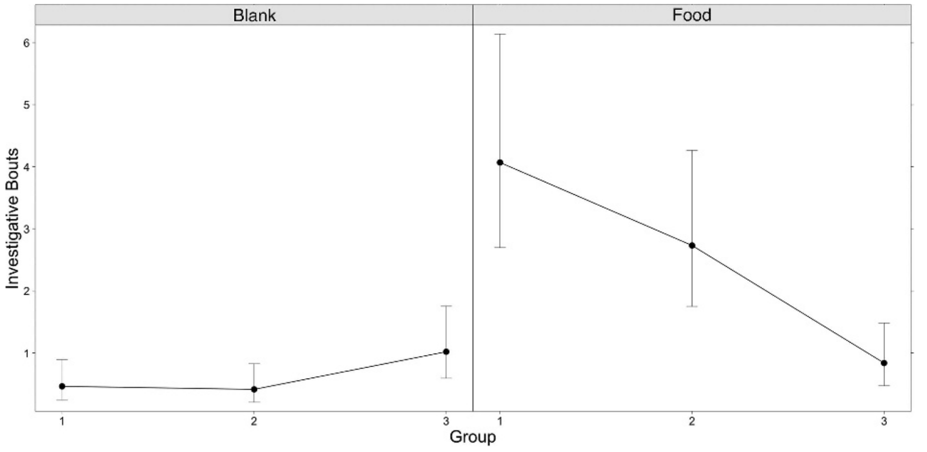

FIGURE 1. Number of investigative bouts by group for desert tortoises (*Gopherus agassizii*) in Nevada, USA: (1 = *Mycoplasma agassizii*–negative and no signs of upper respiratory tract disease [URTD], 2 = *M. agassizii*–positive and no signs of URTD, 3 = *M. agassizii*–positive and symptomatic) and scent presented (blank or food). Means are shown with 95% confidence intervals.

(P=0.068–0.069) for the health status group alone, where group 3 (tortoises with nasal discharge) showed lower investigative behavior. The significance of the interaction was evident in the behavior of the animals, where animals without nasal discharge responded strongly to the containers holding food, while tortoises with discharge showed an overall reduction in investigative behavior, with no difference between scent types (Fig. 1). Tortoises with nasal discharge still had an appetite, as 95% of clinically affected animals ate food that was visually presented to them post-testing. Models with ELISA status alone performed poorly, suggesting that exposure to *M. agassizii* does not interfere with olfaction in the absence of clinical signs.

We speculate that the ability of tortoises to navigate, find mates, and discriminate among conspecifics, all considered to be at least partially dependent on olfaction (Alberts et al. 1994; Graham et al. 1996; Galeotti et al. 2007), could similarly be influenced by nasal discharge. Several studies have suggested that pheromones and olfaction play a part in mating and conspecific discrimination (Alberts et al. 1994). If this is true, the ability of males with nasal discharge to find females and the responsiveness of females toward males may be inhibited by reduced olfaction. This may be exacerbated in low-density populations if the tortoises rely on chemosignals to find one another and could possibly enhance Allee effects in these populations.

Our results suggest that tortoises with nasal discharge may also have difficulty locating food due to a reduced sense of smell. However, if an animal is able to locate food by visual and not olfactory cues, the presence of nasal discharge in the absence of other clinical signs should not impair appetite. Reduced olfactory ability due to nasal discharge may have indirect effects that disadvantage desert tortoises. Further studies are warranted to determine the extent and type of these effects and if breeding success or survival are reduced as a result.

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