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Source: Journal of Wildlife Diseases, 44(4) : 988-991

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-44.4.988

Journal of Wildlife Diseases, 44(4), 2008, pp. 988–991

Salmonella from Gopher Tortoises (*Gopherus polyphemus*) in South Georgia

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ABSTRACT: From 2002 to 2006, gopher tortoises (*Gopherus polyphemus*) were collected at Moody Air Force Base, Lowndes/Lanier counties, Georgia, USA, and opportunistically surveyed for the presence of *Salmonella* species. Four of 155 (2.6%) cloacal swabs collected from 80 tortoises were positive for the presence of *Salmonella enterica*, and the following serovars were identified: Give, Hartford, Javiana, and Luciana. Female tortoises (5%) were infected at a rate similar to male tortoises (5%). All isolates were obtained from adult tortoises (n=73); subadults (n=7) were all negative. Each isolated serovar is a potential human pathogen, suggesting appropriate precautions should be emphasized when handling these animals.

Key words: Gopher tortoise, *Gopherus polyphemus*, *Salmonella*.

Salmonellosis is a major cause of human illness in the United States, with nearly 1.4 million cases and 600 deaths a year (Mead et al., 1999). In the United States, approximately 74,000 cases of *Salmonella* per year are attributed to handling pet reptiles or amphibians (Mermin et al., 2004). Reptile-associated salmonellosis is particularly associated with invasive disease (Cieslak et al., 1994) and can frequently involve children (Mermin et al., 1997). *Salmonella* usually causes a self-limiting gastrointestinal disorder, but it may have severe manifestations, even causing death (Mermin et al., 1997; CDC, 2003).

Reptiles have long been recognized as a source of *Salmonella* infections (Hinshaw and McNeil, 1945; McNeil and Hinshaw, 1946). Pet turtles were a common source of reptile-associated salmonellosis until 1975 when the Food and Drug Administration banned the sale of turtles less than 4 inches long. Direct transmission of *Salmonella* to humans may occur through handling of an infected reptile; indirect transmission may occur through contact with feces or by handling objects contaminated by reptiles. In turtles, *Salmonella* is routinely isolated in healthy animals, although *Salmonella* may cause significant pathology (Gonzalez et al., 2005).

The gopher tortoise (*Gopherus polyphemus*) is the only tortoise to occur in the southeastern United States and as a result of degradation, fragmentation, and loss of sandhill habitat (Bozeman, 1971; Wharton, 1978; Noss, 1989), gopher tortoises are state-listed as threatened or endangered across much of their range (Ernst et al., 1994). Gopher tortoises are considered a keystone organism because their burrows are home to more than 350 species of vertebrates and invertebrates, including several threatened and endangered species, such as the eastern indigo snake (*Drymarchon corais cooperi*), gopher frog (*Rana sevosa*), and Florida mouse (*Podomys floridanus*) (Jackson and Milstrey, 1989; Lips, 1991; Witz et al., 1991). Although long recognized in pet reptiles, the epizootiologic status of *Salmonella* in free-living tortoises is not well known. The objective of this study was to determine the prevalence of *Salmonella* species in a local gopher tortoise population.

From March 2002 to September 2006, gopher tortoises were captured as part of a study to evaluate a population located on Moody Air Force Base, Lowndes/Lanier counties, Georgia, USA (30°58′00.21″N, 83°10′32.70″W) for the presence of *Mycoplasma agassizii*, the causative agent of upper respiratory tract disease. Cloacal swabs were opportunistically collected from tortoises and examined for the presence of *Salmonella*. Captured gopher tortoises were manually restrained, placed with the plastron facing dorsally, and a Cary Blair (Becton Dickinson & Company, Sparks, Maryland, USA) transport media swab was collected from the cloaca. Collected swabs were placed on ice and transported to the laboratory. Cloacal swabs taken in the field were broken off into 10 ml of Selenite F broth (Becton Dickinson & Company) and incubated overnight at 37 C. The next day, 2 XLD (Becton Dickinson & Company) agar plates were streaked for isolation from each tube of broth; plates were incubated overnight at 37 C. Single nonlactose- and nonsucrose-fermenting, black (H_2S-producing) colonies were restreaked to another XLD plate for isolation and further evaluation. If the isolated colonies still demonstrated H_2S production and did not ferment lactose or sucrose, they were streaked on nutrient agar (Becton Dickinson & Company) and inoculated into an Enterotube II for identification. *Salmonella* isolates were submitted to the National Veterinary Services Laboratory (Ames, Iowa, USA) for serotyping.

Eighty unique tortoises were sampled from one to seven times between 2002 and 2006 for a total of 155 cloacal samples. Four tortoises (5%) tested positive at some point during the study. Four (2.6%) cloacal swabs produced *Salmonella enterica* isolates, and the following serovars were identified: Give, Hartford, Javiana, and Luciana. Thirty-nine female tortoises sampled 74 times produced two (3%) isolates: serovars Give and Luciana. Forty-one male tortoises sampled 81 times yielded the remaining two (3%) isolates: serovars Javiana and Hartford. Seventy-three adults sampled 144 times produced all four isolates; seven subadults, sampled 11 times, were all negative. No significant difference was noted (χ^2 = 0.003, $P>0.05$) in male versus female infection rate. No apparent trends were noted with regard to year or month of collection (Table 1). Isolates were obtained in June (2005, serovar Give), August (2002, serovar Javiana; 2006, serovar Hartford), and September (2005, serovar Luciana). No *Salmonella*-positive animals were suspect or positive for the presence of *Mycoplasma*.

TABLE 1. Gopher tortoise cloacal swab sample distribution, Moody Air Force Base, Valdosta, Georgia, USA, 2002–2006.

Yr	Tortoise collected	*Salmonella* positive	Serovar(s)
2002	52	1	Javiana
2003	42	0	—
2004	27	0	—
2005	17	2	Give, Luciana
2006	17	1	Hartford

Two factors most likely limited the sensitivity of detecting *Salmonella* in the gopher tortoises: the small amounts of feces collected on the cloacal swabs and the enrichment procedure used. Funk et al. (2000), who used rectal swabs as well as fecal samples of 1, 10, and 25 g in their efforts to culture *S. enterica* from swine, found that the relative sensitivity of detection (number of positive animals for a given fecal sample weight/number positive for all fecal sample weights) was directly related to the fecal sample weight. They further determined that the relative sensitivity of detection for rectal swabs was only 8.7% (Funk et al., 2000). Using this estimate, it seems possible that the actual rates of *Salmonella* infection in the gopher tortoises sampled might have been 11 times higher than those reported in the present study. The enrichment procedure used in the present study consisted of incubating the cloacal swabs overnight at 37 C in Selenite F broth. However, in studies with other enrichment broths, Nietfeld et al. (1998) found that adding an additional delayed secondary enrichment (after the primary enrichment) increased the numbers of pig rectal swabs that tested positive for *Salmonella*. For naturally infected animals, only four of 367 pigs were positive for *Salmonella* after a 24-hr enrichment, whereas 10 additional pigs were positive for *Salmonella* after the delayed secondary enrichment (Nietfeld et al., 1998). Based on these data, one can estimate that the actual rates of *Salmonella* infection in the gopher tortoises sampled might have been 3 times higher than those reported in the present study. Thus, it seems likely that the calculated *Salmonella* infection rates reported in the present study markedly underestimate the actual prevalence of *Salmonella* infection in the gopher tortoises sampled.

Salmonella has been recorded frequently in both terrestrial and aquatic chelonians. Previous studies have ranged from zero prevalence in several studies to 100% in *Testudo graeca* in Spain (Hidalgo-Vila et al., 2007). Among land-dwelling tortoises in the United States, *Salmonella* species have been isolated previously from 5.1% of 413 desert tortoises (*Gopherus agassizii*; Dickinson et al., 2001). The present study represents one of the few times wild tortoises have been surveyed repeatedly for the presence of *Salmonella*. Interestingly, each of the four positive tortoises was sampled on multiple occasions (up to seven times); one of the four positives (serovar Javiana) had a subsequent negative sample 2 yr later.

Serovars isolated in this study have been reported previously in human salmonellosis cases. Serovar Give is commonly found in animals, but it is rarely found in humans (Higgins et al., 1997). Serovar Javiana was among the 15 most common serovars isolated in the United States during 1987–1997 (Olsen et al., 2001), it was responsible for at least one outbreak of salmonellosis in Georgia in 2000 (Georgia Department of Human Resources, Division of Public Health, 2001), and it was documented from at least 56 individuals in Georgia in 2004 (Georgia Department of Human Resources, Division of Public Health, 2005). Serovar Hartford was among the top 20 increasing serovars in the United States during 1987–1997 (Olsen et al., 2001), and serovar Luciana is rarely reported from humans (CDC, 2004).

The range of different *Salmonella* serovars detected in this study is similar to results from other studies (Brianes et al., 2004; Corrente et al., 2004). Although the four serovars isolated are documented human pathogens, the extent to which gopher tortoises serve as carriers of potentially zoonotic *Salmonella* remains unknown. Gopher tortoises are generally reclusive animals that spend significant periods sheltered in their burrows, suggesting limited human contact. The potential for human origin of *Salmonella* is unclear, although the military mission at Moody Air Force Base requires field training in gopher tortoise habitat, suggesting a human-influenced environment. These serovars may represent those from the environmental niche, habitat and/or diet components of the gopher tortoise (Brianes et al., 2004). Results presented suggest that those individuals who handle wild tortoises should emphasize sanitary precautions.

We thank P. Barber, M. Brown, R. Shaw, and A. Evans for assistance with collection of samples. This work was supported by Department of Defense grant DAMD17-00-2-0034.

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Received for publication 17 May 2007.