MYCOPLASMA AGASSIZII IN MORAFKA’S DESERT TORTOISE (GOPHERUS MORAFKAI) IN MEXICO

Kristin H. Berry,1,7 Mary B. Brown,2 Mercy Vaughn,3 Timothy A. Gowan,1,6 Mary Ann Hasskamp,4 and Ma. Cristina Meléndez Torres5

1 US Geological Survey, Western Ecological Research Center, 21803 Cactus Avenue, Suite F, Riverside, California 92518, USA
2 Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611-0880, USA
3 179 Niblick Road, PMB 272, Paso Robles, California 93446, USA
4 PO Box 765, Challis, Idaho 83226, USA
5 Comisión de Ecología y Desarrollo Sustentable del Estado de Sonora, Reyes y Aguascalientes esq. S/N, Col. San Benito, C.P. 83190, Hermosillo, Sonora, Mexico
6 Current address: Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 8th Ave. SE, St. Petersburg, Florida 33701, USA
7 Corresponding author (email: kristin_berry@usgs.gov)
MYCOPLASMA AGASSIZII IN MORAFKA’S DESERT TORTOISE (GOPHERUS MORAFKAI) IN MEXICO

Kristin H. Berry,1,7 Mary B. Brown,2 Mercy Vaughn,3 Timothy A. Gowan,1,6 Mary Ann Hasskamp,4 and Ma. Cristina Meléndez Torres5

1 US Geological Survey, Western Ecological Research Center, 21803 Cactus Avenue, Suite F, Riverside, California 92518, USA
2 Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611-0880, USA
3 179 Niblick Road, PMB 272, Paso Robles, California 93446, USA
4 PO Box 765, Challis, Idaho 83226, USA
5 Comisión de Ecología y Desarrollo Sustentable del Estado de Sonora, Reyes y Aguascalientes esq. S/N, Col. San Benito, C.P. 83190, Hermosillo, Sonora, Mexico
6 Current address: Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 8th Ave. SE, St. Petersburg, Florida 33701, USA
7 Corresponding author (email: kristin_berry@usgs.gov)

ABSTRACT: We conducted health evaluations of 69 wild and 22 captive Morafka’s desert tortoises (Gopherus morafkai) in Mexico between 2005 and 2008. The wild tortoises were from 11 sites in the states of Sonora and Sinaloa, and the captive tortoises were from the state-managed Centro Ecológico de Sonora Zoo in Hermosillo and a private residence in the town of Alamos. We tested 88 tortoises for mycoplasmal upper respiratory tract disease (URTD) using enzyme-linked immunosorbent assays for specific antibody and by culture and PCR for detection of Mycoplasma agassizii and Mycoplasma testudineum. Fifteen of 22 captive tortoises had one or more positive diagnostic test results for M. agassizii whereas no wild tortoises had positive tests. Tortoises with positive tests also had significantly more moderate and severe clinical signs of mycoplasmosis on beaks and nares compared to tortoises with negative tests. Captive tortoises also exhibited significantly more clinical signs of illness than did wild tortoises, including lethargy and moderate to severe ocular signs. The severity of trauma and diseases of the shell and integument did not differ significantly among tortoises by site; however, clinical signs of moderate to severe trauma and disease were more prevalent in older tortoises. Similar to research findings for other species in the genus Gopherus in the US, we found that URTD is an important disease in captive tortoises. If they escape or are released by intention or accident to the wild, captive tortoises are likely to pose risks to healthy, naïve wild populations.

Key words: Cutaneous dyskeratosis, Gopherus morafkai, health evaluations, Morafka’s tortoise, Mycoplasma agassizii, Mycoplasma testudineum, trauma.

INTRODUCTION

The recently described Morafka’s desert tortoise (Gopherus morafkai) is part of a species complex that includes Gopherus agassizii (Murphy et al. 2011). The geographic range of G. morafkai extends from the Colorado River in Arizona south into Sinaloa, Mexico (Murphy et al. 2011) and now encompasses the former southern two-thirds of the range of G. agassizii. Gopherus morafkai is a candidate species for federal listing as threatened in the US (US Fish and Wildlife Service [USFWS] 2013) and is federally listed as “amenazada,” or threatened, under its former name, G. agassizii, in Mexico (Secretaría de Medio Ambiente Y Recursos Naturales [SEMARNAT] 2010). Little is known about populations of G. morafkai in Mexico (USFWS 2010) compared with G. agassizii.

New and emerging diseases associated with population declines contributed to federal listing of G. agassizii as a threatened species (USFWS 1990) and brought attention to the topic for chelonians in general (Jacobson et al. 1991). Upper respiratory tract disease (URTD) caused by one or more species of Mycoplasma is among the best characterized of reptilian diseases (Jacobson et al. 2014). Two species, Mycoplasma agassizii (Brown et al. 1994, 2004) and Mycoplasma testudin-
neum (Brown et al. 2001), were identified, and enzyme-linked immunosorbent assays (ELISAs) were developed for rapid detection of anti-Mycoplasma antibodies in plasma (Wendland et al. 2007). Other contributors to poor health and disease include starvation and dehydration (Berry et al. 2002); cutaneous dyskeratosis (Jacobson et al. 1994); shell necrosis, urolithiasis, sarcocystosis, renal and polyarticular gout, mycotic pneumonia (Homier et al. 1998); oxalosis (Jacobson et al. 2009); elemental toxicosis (Seltzer and Berry 2005); and herpesvirus (Jacobson et al. 2012).

Far less is known about health and diseases in G. morafkai. In the geographic range of G. morafkai in Arizona, US, wild G. morafkai have been evaluated for general health at two sites (Dickinson et al. 2001, 2002a, 2002b). Both wild and captive G. morafkai were tested for URTD caused by M. agassizii in the urban areas of Kingman, Phoenix, and Tucson and at remote sites within the Sonoran Desert (Dickinson et al. 2005; Jones 2008).

Our objectives were to determine general health, distribution, and prevalence of mycoplasmal URTD, diseases of the integument and shell, other diseases (e.g., herpesvirus), and trauma in wild and captive G. morafkai populations in Mexico. Our study is part of a large, multiyear project to determine the status, distribution, genetics, and ecology of G. morafkai in Mexico.

MATERIALS AND METHODS

Study sites and tortoise evaluations

We established 11 study sites for wild tortoises at private and cooperative ranches throughout Mexico and sampled captive tortoises at two sites: the state-managed Centro Ecológico de Sonora (CES) Zoo in Hermosillo and a private residence in the town of Alamos, Sonora (Fig. 1 and Table 1). Tortoises occurred in the Plains of Sonora in the Hermosillo Region (Turner and Brown 1982), Sinaloan Thornscrub in the Obregón Region (Brown 1982), and Sinaloan Dry Deciduous Forest in the Alamos Region and in the state of Sinaloa (Gentry 1982; Brown et al. 2007).

Teams of biologists collected data on wild tortoises 8–18 November 2005, 9–16 September 2006, 14–18 November 2006, and 6–10 November 2008. On 12 November 2005, a team assessed two captives kept both inside and outside a residence in Alamos. On 16 November 2005, a team assessed 20 captive tortoises at the CES Zoo whose provenance and medical histories were unknown; the captives were housed together in large groups.

Each tortoise was processed using a standard protocol that included identification with a unique number on 4 × 10-mm paper applied with epoxy to a posterior costal scute (wild tortoises only) and digital photography of the face, eyes, beak, nares, limbs, tail, plastron, and carapace (Berry and Christopher 2001). A few tortoises were notched on marginal scutes. Field teams assessed sex and measured carapace length (MCL, mm) at the midline and mass (g) (spring scales, precision 3%; Pesola®, Baar, Switzerland) to determine mass-length relationships (Jacobson et al. 1993) and general body condition (Berry and Christopher 2001).

Using field data sheets and digital images, we evaluated each tortoise for clinical signs of disease or abnormal behavior (e.g., irregular gait, lack of responsiveness, lethargy), trauma, and other lesions (Berry and Christopher 2001; Berry et al. 2002). Based on examination of eyes, nares, beak, mouth, and forelegs we
Table 1. Numbers of health evaluations conducted on captive and wild Morafka's desert tortoises (*Gopherus morafkai*) at 13 sites in Mexico, arrayed by site, size class, and sex.

<table>
<thead>
<tr>
<th>Status and location of tortoises</th>
<th>Coordinates</th>
<th>Year</th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Captive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hermosillo Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centro Ecológico de Sonora Zoo</td>
<td>29°0'58.68&quot;N, 110°57'3.5994&quot;W</td>
<td>2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alamos Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captive tortoises, subtotal</td>
<td></td>
<td></td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hermosillo Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rancho La Pintada</td>
<td>28°35'41.9994&quot;N, 110°57'50.3994&quot;W</td>
<td>2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rancho San Judas</td>
<td>29°21'46.7994&quot;N, 111°7'4.7994&quot;W</td>
<td>2006</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rancho Cerro Colorado</td>
<td>28°46'24.5994&quot;N, 110°53'56.4&quot;W</td>
<td>2006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obregón Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rancho El Chupadero</td>
<td>27°56'54.5994&quot;N, 110°0'7.1994&quot;W</td>
<td>2006</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Rancho Moscobampo</td>
<td>28°14'25.44&quot;N, 110°26'20.3994&quot;W</td>
<td>2006</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Alamos Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rancho Las Cabras</td>
<td>27°0'26.28&quot;N, 108°54'25.1994&quot;W</td>
<td>2005</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Sinaloa Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Miguel</td>
<td>26°0'0&quot;N, 109°1'51.6&quot;W</td>
<td>2008</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rancho Carricito Topolobampo</td>
<td>25°31'37.2&quot;N, 108°58'33.5994&quot;W</td>
<td>2008</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rancho Cajo de Félix</td>
<td>26°31'22.7994&quot;N, 108°24'34.9994&quot;W</td>
<td>2008</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Wild tortoises, subtotal</td>
<td></td>
<td>5</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>

* (-) = no data.
noted clinical signs of URTD, which can be caused by *Mycoplasma* spp., herpesvirus, or other pathogens (Jacobson et al. 1991; Brown et al. 1994; Christopher et al. 2003). The most common, although not specific, clinical sign for mycoplasmosis is a purulent nasal discharge (Jacobson et al. 1995); other signs are occluded or partially occluded nares, crusts or staining on the beak or around the nares, dried mucus on the beak, and eroded nares. Ocular signs include edema of the palpebrae and periocular area, crusts, ocular discharge, bulging of the globe, and mucus. When possible, we examined the oral cavity for tissue color and signs of plaques or ulcers for herpesvirus (Jacobson et al. 2012). We rated variables on the eyes, nares, and beak for severity of clinical signs using four classes: normal, mild, moderate, or severe following Berry and Christopher (2001). We assessed lesions on the integument from disease (e.g., necrosis or cutaneous dyskeratosis; Jacobson et al. 1994; Homer et al. 1998). Cutaneous dyskeratosis is characterized by discoloration and deterioration of laminae on scutes and, in severe cases, exposure of bone and soft tissue. We graded shell lesions and trauma on head, limbs, tail, carapace, and plastron according to distribution and extent (1 = none, 2 = <10%, 3 = 10–40%, and 4 = >40%), severity (1 = no sign, 2 = mild, 3 = moderate, and 4 = severe), and chronicity (not active, active, healed). For trauma, we categorized causes of injury (e.g., gunshot [Berry 1986], predator attacks from corvids [Bourman 1993] or mammalian carnivores).

We collected ~1 cc of blood from the subcarapacial or brachial veins of tortoises ≥150 mm MCL (Hernandez-Divers et al. 2002). Plasma was tested for specific antibodies using ELISAs for *M. agassizii* (Wendland et al. 2007) and *M. testudineum* (Brown et al. 1995, 2004). The ELISA for *M. testudineum* was limited to tortoises evaluated in 2005 (n = 50) and 2008 (n = 6). We also obtained nasal lavages from all tortoises for culture and PCR (Brown et al. 2002). *Mycoplasma* species were determined based on a unique restriction fragment-length polymorphism fingerprint of the PCR amplicon (Brown et al. 2002; Wendland et al. 2007). The ELISA results were reported as end-point titers (EPT) and samples were classified by EPTs of <32 as negative, 32 as suspect, 64 as low positive, 128 as medium positive, and 256 as high positive for *Mycoplasma* spp. antibody (Wendland et al. 2007). We chose ELISA to detect specific antibody because, unlike Western blot using a single antigen (Hunter et al. 2008), the ELISA is strain-independent and has significantly fewer false-negative results (Wendland et al. 2010).

**Data analysis**

We classified tortoises as juvenile or immature (<179 mm MCL) or adult (≥179 mm MCL) using data on size at first reproduction for female *G. agassizii* (~180 mm MCL: Berry and Christopher 2001). We used this classification with caution for *G. morafkai* because the smallest *G. morafkai* with eggs from Arizona was 220 mm MCL (Averill-Murray 2002). Regional differences in size at first reproduction may represent a character difference in taxa within the desert tortoise complex (Murphy et al. 2011). We assigned tortoises ≥179 mm MCL to one of five relative adult age classes (very young, young, middle-aged, old, and very old) based on appearance of scutes—specifically presence and wear of areola and growth rings on laminae, age-related thickness of bone and scute, and age-related depressions on the scutes as criteria (Berry and Woodman 1984; Christopher et al. 2003).

Because mass-length relationships for *G. morafkai* differ by sex and site (Dickinson et al. 2002a), we used analysis of covariance (ANCOVA) to test significance of mass differences between sexes with MCL as the covariate. We also tested mass differences between captive and wild tortoises after controlling for MCL with separate ANCOVAs for each sex. We then developed regressions of mass on MCL for all tortoises ≥179 mm MCL with separate 95% confidence intervals (CI) for captive and wild tortoises.

We analyzed for agreement between results of diagnostic tests and clinical signs. For each combination of two diagnostic tests and clinical signs (ELISA and PCR for *M. agassizii*, presence of nasal discharge), we divided the number of tortoises in which the tests gave the same results, either positive or negative or present or absent, by the total number of tortoises evaluated by the diagnostic test and clinical signs. Three tortoises with ELISA-suspect results and one tortoise with no data for the ELISA (total = 4) were excluded from analyses involving ELISA results. The tortoises with *M. testudineum* were excluded from agreement tests because none had positive laboratory tests.

Using Fisher’s exact test, we compared ELISA and culture results among sites and with severity of different types of clinical signs. We also used Fisher’s exact test to compare captive with wild tortoises on the ELISA results for *M. agassizii* and for ratings of...
clinical signs (none or mild vs. moderate to severe) typical of URTD in the eyes, beak, and nares. Similarly, we compared captive and wild tortoises for clinical signs (none or mild vs. moderate to severe) of lesions and trauma of the integument and shell. Statistical analyses were performed using SYSTAT 12.0 (SYSTAT Software, Inc., Richmond, California, USA).

RESULTS

Sixty-nine wild tortoises and 20 captives at the CES Zoo and two captives at a residence in Alamos were evaluated (Table 1). One of the 69 wild tortoises had red paint on the carapace, indicating it had been handled and was a released captive. The size class composition of the tortoises was five juvenile or immature tortoises (110–167 mm MCL) and 47 male and 39 female adult tortoises (males, 182–284 mm; females, 179–286 mm). Using the relative age classes for 86 adult tortoises (males, 182–284 mm; females, 179–286 mm), the prevalence of mycoplasmal URTD between captive and wild tortoises (Fig. 3 and Tables 2, 3) was marked. No wild tortoises were positive by ELISA or PCR for either M. agassizii or M. testudineum antibody or DNA. Prevalence of M. agassizii by ELISA, culture, or both differed among the 13 sites (P=0.001, P=0.001, respectively). All tortoises with positive tests (68%, 15/22) were captives at the CES Zoo. Positive results were obtained by both ELISA and PCR in 50% (9/18) of CES Zoo captives. Clinical disease (nasal discharge, including wet nares, the most-common clinical sign of URTD) was present in 55% (12/22) of captives. Only five captive tortoises were either males (ANCOVA, F1,44=0.589, P=0.447) or females (ANCOVA, F1,36=0.252, P=0.619; Fig. 2). Therefore, we grouped both sexes for subsequent analyses. Several wild and captive tortoises were outside the 95% CIs for mass-length relationships; some were low in mass for length whereas others were higher (Fig. 2). One low mass captive was emaciated.

Diagnostic tests and clinical signs

The disparity in evidence of mycoplasmal URTD between captive and wild tortoises (Fig. 3 and Tables 2, 3) was marked. No wild tortoises were positive by ELISA or PCR for either M. agassizii or M. testudineum antibody or DNA. Prevalence of M. agassizii by ELISA, culture, or both differed among the 13 sites (P=0.001, P=0.001, respectively). All tortoises with positive tests (68%, 15/22) were captives at the CES Zoo. Positive results were obtained by both ELISA and PCR in 50% (9/18) of CES Zoo captives. Clinical disease (nasal discharge, including wet nares, the most-common clinical sign of URTD) was present in 55% (12/22) of captives. Only five captive tortoises were...
negative for both diagnostic tests and for nasal discharge, and two of the five were the captives at the residence in Alamos. At the CES Zoo, three captive tortoises had suspect ELISA results and nasal discharges; one of the three was also PCR positive. Only 9% (6/69) of wild tortoises had nasal discharge.

Because of the disparity between captive and wild tortoises with respect to URTD caused by *M. agassizii*, the agreements among diagnostic tests represent scenarios for two extremes: a population with mostly true negatives and a population with mostly true positives. The overall agreement between ELISA and PCR was 95% for all tortoises tested, with 100% and 78% agreement in the wild and captive populations, respectively (Table 3). The presence of nasal discharge, which is not specific for URTD, had similar or lower agreements with either ELISA or PCR for captives or wild tortoises or both (Table 3). There was no

**Table 2.** Results of diagnostic tests (enzyme-linked immunosorbent assay [ELISA] and culture) for *Mycoplasma agassizii* and clinical signs (nasal discharge) in captive and wild Morafka’s desert tortoises (*Gopherus morafkai*) from Mexico: 20 captives at the Centro Ecológico de Sonora (CES) Zoo in Hermosillo, two captives at a residence in the town of Alamos, and 69 wild tortoises at remote sites. All positive cultures had a DNA fingerprint consistent with *M. agassizii*.

<table>
<thead>
<tr>
<th>Location of tortoises; results of diagnostic tests and clinical observations</th>
<th>Numbers of tortoisesa</th>
<th>ELISA</th>
<th>Culture/DNA fingerprint</th>
<th>Nasal discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES Zoo tortoises</td>
<td>Positive or present</td>
<td>11</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Negative or absent</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Suspect</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>No data</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Captives in Alamos</td>
<td>Negative or absent</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Captive totals</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Wild tortoises</td>
<td>Positive or present</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Negative or absent</td>
<td>67</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>No data</td>
<td>2</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>Wild totals</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>

a (–) = not applicable.

**Table 3.** Agreement between diagnostic tests for captive and wild Morafka’s desert tortoises (*Gopherus morafkai*) from Mexico. Agreement = Number of tortoises for which tests gave the same results, either positive or negative, divided by the total number of tortoises tested by one or more methods. All PCR products gave a DNA fingerprint consistent with *Mycoplasma agassizii*.

<table>
<thead>
<tr>
<th>Diagnostic testsa and clinical signs</th>
<th>No. tests that agreed / No. tests performed (%)b</th>
<th>Captive</th>
<th>Wild</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA and PCR</td>
<td>14/18 (78)</td>
<td>60/60 (100)</td>
<td>74/78 (95)</td>
<td></td>
</tr>
<tr>
<td>ELISA and ND</td>
<td>14/18 (78)</td>
<td>61/67 (91)</td>
<td>75/85 (88)</td>
<td></td>
</tr>
<tr>
<td>PCR and ND</td>
<td>13/22 (59)</td>
<td>54/60 (90)</td>
<td>67/82 (82)</td>
<td></td>
</tr>
<tr>
<td>ELISA and PCR and ND</td>
<td>11/18 (61)</td>
<td>54/60 (90)</td>
<td>65/78 (83)</td>
<td></td>
</tr>
</tbody>
</table>

a ELISA = enzyme linked immunosorbent assay; ND = nasal discharge.
b ELISA results were not obtained for one captive and two wild tortoises; three captive tortoises with ELISA-suspect results were excluded from calculations involving ELISA. PCR results were not obtained for nine wild tortoises.
Fifty-five tortoises were tested for *M. testudineum* and *M. testudineum* antibody. One wild tortoise at La Sierrita and three captive tortoises at the CES Zoo had suspect test results using the ELISA, but PCR tests were negative. Two of the three captive tortoises with suspect results for *M. testudineum* were also ELISA or culture-positive for *M. agassizii*.

In general, tortoises with positive laboratory tests also had clinical signs of illness (Table 4). Significantly more tortoises with positive ELISA results and cultures for *M. agassizii* were lethargic and had significantly more moderate and severe ratings for clinical signs of URTD on the beak and nares. However, there were no significant relationships between positive ELISA and culture results with ocular signs of disease or moderate to severe trauma. Tortoises with positive ELISA results tended to have more moderate to severe shell disease, but the association was not significant.

As a group, captive tortoises exhibited more signs of illness than did wild tortoises. Only CES Zoo captive tortoises exhibited abnormal signs of behavior: lethargy (n = 7), limbs or head hanging loose (n = 7), not alert (n = 2), dragging a limb or barely able to walk (n = 2). Significantly more captives had clinical signs on the beak and nares, and the signs were more severe (absent vs. present and mild vs. moderate/severe: P = 0.001, P = 0.002, respectively). The site differences were statistically significant with the CES Zoo having the highest proportion of tortoises with clinical signs on the beak and nares and having more moderate to severe clinical signs (P = 0.001, P = 0.029, respectively). Captive tortoises also had significantly more moderate to severe ocular signs of disease than did wild tortoises (P = 0.012), but differences were not significant by site (P = 0.294).

### Shell lesions, diseases of the integument, and trauma

Fifteen (17\%) of 91 tortoises had moderate to severe ratings for disease-related lesions to the shell and integument for one or more parts of the body. Nine captive and 16 wild tortoises exhibited pitting, usually on the plastron, and some had areas of blackened and exposed necrotic bone in the pits. Two wild tortoises had evidence of fungus on the shell. Most tortoises (73\%, 66/91) had mild to severe signs of cutaneous dyskeratosis on one or more parts of the body; the lesions were most common on the forelimbs (56\%, 51/91) followed by plastron (42\%, 38/91), head (22\%, 20/91), carapace (19\%, 17/91), and gular horn (14\%, 13/91). When captive and wild tortoises were compared, no statistically

### Table 4. Relationships between positive diagnostic tests (enzyme-linked immunosorbent assay [ELISA] and cultures) and clinical signs of disease for Morafka’s desert tortoise (*Gopherus morafkai*) from Mexico.

<table>
<thead>
<tr>
<th>Clinical signs of disease</th>
<th>ELISA</th>
<th>Cultures/DNA fingerprint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant relationships between ELISA- and culture-positive tortoises</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tortoises were more lethargic</td>
<td>0.006</td>
<td>0.011</td>
</tr>
<tr>
<td>Tortoises had more moderate and severe ratings of beak and nares</td>
<td>0.039</td>
<td>0.001</td>
</tr>
<tr>
<td>Nonsignificant relationships between ELISA- and culture-positive tortoises</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tortoises did not have more moderate to severe ocular signs</td>
<td>0.196</td>
<td>0.124</td>
</tr>
<tr>
<td>Tortoises had nonstatistically significant trend for moderate to severe shell disease</td>
<td>0.098</td>
<td>0.424</td>
</tr>
<tr>
<td>Tortoises did not have more moderate to severe trauma</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>
significant differences existed for disease-related lesions when head, limbs, gular horn, plastron, and carapace were evaluated separately or when analyzed as part of an overall severity rating ($P=0.509$). No significant differences were evident by site for diseases of the shell and integument ($P=0.205$); however, shell disease was significantly more prevalent in older tortoises ($P=0.016$).

Most tortoises had signs of trauma. Signs were mild for 45 tortoises (50%, 45/91) and moderate to severe for 24 tortoises (26%, 24/91). In most cases, the trauma had healed (57%, 52/91) or was in the process of healing (19%, 17/91). More injuries occurred to the carapace (50%, 45/91) than to other body parts followed by the plastron (34%, 33/91), gular horn (22%, 20/91), and head (7%, 6/91). One tortoise had 60% of the lower mandible chewed off, and three tortoises had raw or fresh limb wounds.

Damage to the shell and limbs appeared to be primarily from predator attacks. No statistically significant differences existed between captive and wild tortoises for trauma when analyzed by site ($P=0.146$) or as an overall severity rating or by individual body parts ($P=0.353$). However, older tortoises had more-severe trauma ($P=0.039$).

**DISCUSSION**

The most significant findings from this study of *G. morafkai* are the patterns of distribution and prevalence of mycoplasmal URTD in Sonora and Sinaloa, Mexico. The evaluations of captive and wild tortoises revealed a pattern similar to that reported for *G. morafkai* in the northern half of its geographic range in Arizona: captive tortoises have a high prevalence compared to wild tortoises in remote areas away from frequent human contact (Dickinson et al. 2005; Jones 2008). In Mexico, half of captives were positive for antibody to *M. agassizii*, indicating exposure to this pathogen, and more than half of the captives were culture positive and therefore capable of shedding the bacteria during intraspecific contact. All of the test-positive captives were at the CES Zoo. Because mycoplasmal infections are spread by direct contact between individual tortoises (Brown et al. 1994), the high prevalence implies that a large number of animals were likely infectious in the CES Zoo. Two tortoises had *M. agassizii* isolated from nasal lavages but were ELISA negative. It is likely that these two animals were newly infected and had not yet seroconverted (Jacobson et al. 2014). A third animal had *M. agassizii* isolated from nasal lavage and tested ELISA suspect, suggesting an early-stage infection in an animal starting to produce detectable antibodies. In contrast, no antibody-positive or culture-positive tortoises were found in the 11 wild populations, suggesting that captive populations are likely to pose risks to healthy, naïve wild populations if released into the wild.

Mycoplasmosis is considered a threat to wild *G. agassizii* (Christopher et al. 2003; Jacobson et al. 2014). Captive *G. agassizii* living in desert towns in the central, western, and southern Mojave Desert have a high (82.7%) prevalence of antibody to *M. agassizii* (Johnson et al. 2006). Wild *G. agassizii* living near desert towns and settlements also may be more likely to have mycoplasmosis (Jacobson et al. 1995; Schumacher et al. 1997; Berry et al. 2006). In contrast, tortoises living in remote areas of the Mojave Desert were usually antibody negative (Berry et al. 2006). In the US, thousands of *G. agassizii* and *G. morafkai* live in captivity in the Southwest, and many of these tortoises are now reservoirs for *M. agassizii* and other infectious agents such as herpesvirus (Johnson et al. 2006; Jones 2008; Jacobson et al. 2012). Escape and deliberate release of captives is considered a threat to wild populations and such releases are against federal and state laws (e.g., Arizona State Legislature 2014). Government agencies and academia have developed programs to
alert the public to this threat. In addition, preventive medical protocols are available for management of tortoises and other reptiles housed at zoos or in private collections; these protocols include health assessments and screening, quarantine, long-term monitoring, and necropsies of dead tortoises to determine cause of illness (e.g., Jacobson 1993; Pasmans et al. 2008).

In Mexico, a similar problem with captive *G. morafkai* may have developed. Captive tortoises are common in the city of Hermosillo, and Bury et al. (2002) reported that captives also are being released or translocated. The Mexican government has discouraged the sale and keeping of pet tortoises (Meléndez Torres 1998; Bury et al. 2002). Nevertheless, the free-ranging tortoise with red paint on the carapace found at Rancho La Sierrrita during this study indicates that tortoises are either being handled in the wild or collected, held in captivity, and then released.

The health status of a desert tortoise, specifically in terms of mycoplasmosis, is not necessarily easy to determine. This disease is chronic and animals express clinical signs intermittently. Clinical signs may signal clinical or subclinical disease or they may be absent in an ill tortoise. Jacobson et al. (1995) evaluated the relationships between clinical signs, results of ELISA and cultures, and histopathology. They reported that all tortoises with clinical signs (nasal discharge) of URTD were antibody positive, but five of eight tortoises with no clinical signs and with mild to severe lesions in their nasal cavities were also antibody positive. Three tortoises with mild lesions in the nasal cavity were antibody negative. Thus, histologic lesions typical of mycoplasmosis were present in tortoises with no clinical signs, indicating the presence of subclinical disease. Schumacher et al. (1997) evaluated the relationship between clinical signs of URTD and the ELISA for *M. agassizii*. They found that 93% of tortoises with mucous nasal discharge were antibody positive and that presence of nasal discharge was highly predictive for exposure to *M. agassizii*. They also noted that the ELISA detected potential subclinical infections in 34% of tortoises without clinical signs. In our study, we evaluated clinical signs on the head and beak. Significantly more tortoises with antibody to *M. agassizii* exhibited moderate to severe clinical signs on the beak and nares than did antibody-negative tortoises; the relationship between presence of detectable antibody and moderate to severe ocular signs was not significant, however.

Several potential explanations exist in cases when diagnostic tests are not in agreement with each other or with typical clinical signs such as nasal discharge (e.g., early vs. late stage infection, intermittent expression of clinical signs, and presence of other infectious agents [Brown et al. 1999; Jacobson et al. 2014]). For example, in our study three tortoises at the CES Zoo had suspect ELISA antibody results and nasal discharges. Nasal discharges often precede seroconversion and may be present early in the infection process. One of the three also had a positive PCR. In this particular case—at a site where most tortoises have positive diagnostic tests—the three suspect ELISA results likely represented recent seroconversion and were true indicators of infection (Brown et al. 1999).

Tortoises with lesions of the integument and shell typical of cutaneous dyskeratosis (Jacobson et al. 1994; Homer et al. 1998) were observed both at the CES Zoo and wild sites. This disease has been associated with high mortality rates in *G. agassizii* at some sites in the eastern Mojave and western Sonoran deserts and may be due to nutrient deficiencies or heavy metal toxicosis (Jacobson et al. 1994; Christopher et al. 2003). In Mexico, 73% of *G. morafkai* had clinical signs of cutaneous dyskeratosis compared with 0% to 65.2% at 13 sites in the northern part of the geographic range in Arizona (Dickinson
et al. 2002b) and with 85.4% in a study of G. agassizii at three sites in the Mojave Desert (Christopher et al. 2003). At these latter study sites in the Mojave Desert, hyperglobulinemia, positive M. agassizii cultures, and oral lesions were significantly associated with shell disease (Christopher et al. 2003). At the Mexican sites, most G. morafkai were in the young adult age classes compared with populations studied in the Mojave Desert (Christopher et al. 2003). Disease-related lesions increased with increasing age of G. morafkai, similar to results reported by Christopher et al. (2003) for G. agassizii. Another similarity to the Christopher et al. (2003) study was our finding that tortoises with positive serology for M. agassizii tended to show an association with more-severe shell disease. More tortoises, 77% in our study, showed signs of current or past trauma compared with 41.7% of G. agassizii at three sites in the Mojave Desert, however (Christopher et al. 2003). In the future, continued monitoring for prevalence of infectious agents, including herpesvirus, will be important for conservation of this threatened species.

ACKNOWLEDGMENTS

This project was only possible through the generosity and assistance of many individuals and agencies. We thank F. R. Méndez de la Cruz of the Instituto de Biología, Universidad Nacional Autónoma de México, Cd. México; the Centro Ecológico de Sonora; Comisión de Ecología y Desarrollo Sustentable del Estado de Sonora; Área de Protección de Flora y Fauna Sierra de Álamos–Río Cuchujaqui (CONANP); Secretaría de Medio Ambiente Y Recursos Naturales (SEMARNAT); and owners and members of the following cooperatives and ranches (Rancho): Las Cabras, La Sierrita, El Divisadero, La Pintada, Cerro Colorado, San Miguel, Carricito Topolobampo, and Cajiön de Félix. We thank J. Yee, K. Nussear, K. Phillips, and anonymous reviewers who contributed to improvements in the manuscript. W. Hasskamp, C. Furman, E. Smith, J. Weidensee, K. Herbinson, R. Woodard, L. Pavliscak, T. Poole, P. Woodman, P. Frank, P. Rosen, A. Karl, M. Figueroa, J. Miranda, C. Sánchez, and Y. León assisted in the field. J. Mack assisted with data analysis. M.C.M.T. held federal permits from SEMARNAT. The Mycoplasma Laboratory (M.M.B.), University of Florida, Gainesville, processed all blood and nasal lavage samples (University of Florida, approved Institutional Animal Care and Use Committee protocol A616). The project was supported by the US Geological Survey, University of Florida, University of Arizona, Desert Tortoise Council, Tucson Herpetological Society, Arizona Game and Fish Department, Royal Ontario Museum, and the volunteer field biologists. Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the US government.

LITERATURE CITED


United States and Northwestern Mexico. University of Utah Press, Salt Lake City, Utah.


Jacobson ER, Berry KH, Wellehan JFX Jr, Orrig F, Childress AL, Braun J, Schrenzel M, Yee J,


USFWS. 2010. Endangered and threatened wildlife and plants; 12 month finding on a petition to list the Sonoran population of the desert tortoise as endangered or threatened. *Federal Register* 75:78094–78146.

USFWS. 2013. Endangered and threatened wildlife and plants: Review of native species that are candidates for listing as endangered or threatened; Annual notice of findings on resubmitted petitions; Annual description of progress on listing actions. *Federal Register* 78:70104–70162.


Submitted for publication 2 April 2014. Accepted 22 August 2014.