Upper Respiratory Tract Disease in the Gopher Tortoise Is Caused by Mycoplasma agassizii†

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Upper respiratory tract disease (URTD) has been observed in a number of tortoise species, including the desert tortoise (Gopherus agassizii) and the gopher tortoise (Gopherus polyphemus). Clinical signs of URTD in gopher tortoises are similar to those in desert tortoises and include serous, mucoid, or purulent discharge from the nares, excessive tearing to purulent ocular discharge, conjunctivitis, and edema of the eyelids and ocular glands. The objectives of the present study were to determine if Mycoplasma agassizii was an etiologic agent of URTD in the gopher tortoise and to determine the clinical course of the experimental infection in a dose-response infection study. Tortoises were inoculated intranasally with 0.5 ml (0.25 ml/nostril) of either sterile SP4 broth (control group; n = 10) or 10⁵ color-changing units (CCU) (total dose) of M. agassizii 723 (experimental infection group; n = 9). M. agassizii caused clinical signs compatible with those observed in tortoises with natural infections. Clinical signs of URTD were evident in seven of nine experimentally infected tortoises by 4 weeks postinfection (p.i.) and in eight of nine experimentally infected tortoises by 8 weeks p.i. In the dose-response experiments, tortoises were inoculated intranasally with a low (10⁴ CCU; n = 6), medium (10⁵ CCU; n = 6), or high (10⁶ CCU; n = 5) dose of M. agassizii 723 or with sterile SP4 broth (n = 10). At all time points p.i. in both experiments, M. agassizii could be isolated from the nares of at least 50% of the tortoises. All of the experimentally infected tortoises seroconverted, and levels of antibody were statistically higher in infected animals than in control animals for all time points of >4 weeks p.i. (P < 0.0001). Control tortoises in both experiments did not show clinical signs, did not seroconvert, and did not have detectable M. agassizii by either culture or PCR at any point in the study. Histological lesions were compatible with those observed in tortoises with natural infections. The numbers of M. agassizii 723 did not influence the clinical expression of URTD or the antibody response, suggesting that the strain chosen for these studies was highly virulent. On the basis of the results of the transmission studies, we conclude that M. agassizii is an etiologic agent of URTD in the gopher tortoise.

Gopher tortoises (Gopherus polyphemus) are found in the southeastern United States, with the major population concentrations found in Florida and southern Alabama and Georgia and only remnant populations found in South Carolina, Mississippi, and Louisiana (9). The gopher tortoise is legally protected in all states within the range (Alabama, Mississippi, Louisiana, Georgia, South Carolina, and Florida) and is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, which requires permits for the exportation of the species from the United States to any signatory nation or for reexportation (20). Gopher tortoises are an important element in the ecosystems in which they are found and are considered by many ecologists to be a keystone species. Gophers are the most fossorial of the four North American species of tortoises, digging burrows that may extend 5 m down from the surface and 15 m in length (9, 12). The burrows provide a microclimatically stable environment not only for the tortoises but also for numerous commensal species. Approximately 60 vertebrate species, from snakes to birds, and over 300 invertebrates including spiders, crickets, and beetles have been found in tortoise burrows or have been observed using them as permanent homes or refuges from heat, cold, fire, and predators (14, 22, 36). Several species that either exclusively or frequently use tortoise burrows have legal protection in Florida and other parts of their ranges (6). Thus, tortoises are of critical importance to the ecosystem.

Upper respiratory tract disease (URTD) has been observed in a number of tortoise species (15, 16, 19), including the desert tortoise (Gopherus agassizii) and the gopher tortoise. Clinical signs of URTD have been observed in a number of imported captive tortoise species (19) and in tortoises submitted to the Veterinary Medical Teaching Hospital (VMTH), University of Florida (UF), including the red-footed tortoise (Geochelone carbonaria), leopard tortoise (Geochelone pardalis), Indian star tortoise (Geochelone elegans), and radiated tortoise (Geochelone radiata). Numerous wild and captive gopher tortoises have been submitted to VMTH with clinical signs consistent with URTD.

Clinical signs of URTD in gopher and desert tortoises are similar and include serous, mucoid, or purulent discharge from the nares, excessive tearing to purulent ocular discharge, conjunctivitis, and edema of the eyelids and ocular glands (16, 31). Individual infected tortoises vary in the suite of signs that they have, and the severity can vary from day to day. Nares may become occluded with cascious exudate, preventing externally visible nasal discharge. Tortoises may become lethargic and...
anorectic, leading to dehydration, emaciation, and eventual death from cachexia.

In a previous study, we fulfilled Koch’s postulates and demonstrated that an etiologic agent for URTD in the desert tortoise is Mycoplasma agassizii proposed sp. novum (2, 4). Histologically, the lesions from experimentally infected desert tortoises were consistent with those seen in naturally infected tortoises (4, 16). In the desert tortoise, we have shown that the presence of clinical signs of URTD was positively related to the presence of specific antibody to M. agassizii (31). Additional work is in development of a PCR test for detection of the bacteria in nasal lavage and swab samples (2).

We isolated M. agassizii from the nasal passages of clinically ill gopher tortoises submitted to VMTH, UF. The similarity of the clinical signs and histological lesions between experimentally infected and naturally infected tortoises and the isolation of M. agassizii from naturally infected gopher tortoises suggested that URTD in this species might also be of mycoplasmal origin. The objectives of this study were to determine if M. agassizii was an etiologic agent of URTD in the gopher tortoise and to determine the clinical course of the experimental infection in a dose-response infection study.

**MATERIALS AND METHODS**

**Tortoises.** Gopher tortoises were transferred under Florida Game and Fresh Water Fish Commission permit nos. WX93227, issued to E. R. Jacobson, and WX94037, issued to M. B. Brown, from a development site in central Florida in April, July, and August 1994 and April 1995 and were processed on the day following arrival. Tortoises were examined for clinical signs of URTD: nasal and ocular discharge, palpebral edema, and conjunctivitis. Tortoises were weighed to the nearest 10 g and ketamine hydrochloride (Ketaset; Fort Dodge Laborato-
ries, Inc., Fort Dodge, Iowa) was administered at 20 mg/kg of body weight. A blood sample (2 to 3 ml) was drawn from the jugular or brachial vein and was centrifuged, and an aliquot of plasma was obtained and streaked onto SP4 agar plates (33). Additional blood sample (2 to 3 ml) was centrifuged, and an aliquot of plasma was used for analysis of specific antibody to M. agassizii. Blood was centrifuged, and an aliquot of plasma was used for analysis of specific antibody to M. agassizii. Blood was centrifuged, and an aliquot of plasma was used for analysis of specific antibody to M. agassizii.

**Postinfection monitoring of tortoises.** The tortoises were observed from 3 to 7 days after infection, with some data collected the following week, including multiple objective measures of individual behavior patterns, not every tortoise was observed at each daily observation point. At specific time points (usually 2- to 4-week intervals, depending on the study), tortoises were captured by hand or with wire cage-type traps (Tomahawk Live Trap Company, Tomahawk, Wis.) that were covered with brown paper to protect the animals from the weather. The traps were cleaned, sprayed with bleach solution, and allowed to air dry following each use. The paper was discarded, and fresh paper was used for the next trapping effort. Each tortoise included in a plastic, labeled container (Lindgren Corporation, Watertown, Wis.) for transport and holding. Containers were bleached, scrubbed, and washed in an automatic cage washer before reuse. Tortoises were examined for clinical signs of URTD: nasal and ocular discharge, palpebral edema, and conjunctivitis. A photographic record consisting of right, left, and full face views was made for each tortoise at each time point when the tortoises were captured. The signs were graded individually on a scale of 0 to 3, which indicated none, minimal, mild, and severe signs, respectively. Visual grading of signs was confirmed by independent observation of the photographic record. Serum was obtained for quantitation of specific antibody. Nasal swabs and lavages were obtained for culture and PCR testing.

**Culture.** Mycoplasmal cultures were performed as described previously (4). A 100-μl aliquot of the lavage sample was used for PCR analysis; the remaining sample was serially diluted 10-fold to 10-2 and was incubated at 30°C for a maximum of 3 weeks or until it was determined to be positive or contaminated. In some cases, an aliquot of the broth culture of both lavages and swabs was removed after 24 to 48 h and was used for PCR to confirm growth of M. agassizii. Twenty microtiter of each dilution was placed on SP4 agar, and the plates were incubated at 30°C in 5% CO2. The swabs were streaked onto the surface of an SP4 plate. The plates were examined regularly for a maximum of 6 weeks to detect the growth of mycoplasma.

**PCR.** Nasal aspirate lavage specimens were analyzed for the presence of M. agassizii DNA on the basis of PCR amplification of the 16S rRNA gene (2). Nasal lavage specimens and selected culture samples obtained at between 24 and 48 h were centrifuged at 16,000 × g for 60 min at 4°C, and the supernatant was aspirated. The pellets were resuspended in 3 to 4 μl of 20 mg of proteinase K (Sigma, St. Louis, Mo.) per ml in 20 μl of lysis buffer (100 mM Tris [pH 7.5], 6.5 mM potassium thiocyanate, 0.05% Tween 20), and the mixture was incubated at 37°C for 8 h. After treatment with 1 mg of Proteinase K at 97°C for 5 min, the sample was removed and was added to 45 μl of a reaction solution containing two primers for the 16S rRNA gene at 1 μM each, 25 mM MgCl2, and 2.5 μM of Taq polymerase (Promega, Madison, Wis.). The primers were complementary to sequences found in the V3 variable region of the 16S rRNA gene (sense strand nucleotides [nt] 471 to 490 [5'-CCU TATATTATGACCGTGACTG-T-3'] and a Mycoplasma genus-specific region (anti-sense strand nt 1035 to 1051 [5'-TGACCATGCTGCACTGTGAACCTCT-3']). The samples were subjected to 50 cycles of 1 min at 94°C, primer annealing for 1 min at 55°C, and polymerization for 1 min at 72°C, followed by 10 min at 72°C. Positive samples yielded 576-bp products that were visualized by combining 15 μl of product with 2 μl of bromophen blue in 50 μl of deionized water. Negative samples showed 250-bp and 150-bp bands. A 50-bp Agarose gels in Tris-borate-EDTA buffer. Positive control samples with 250 ng of purified M. agassizii DNA as the template and negative control samples with water in place of a template were included with each amplification run. A molecular weight marker, a HaelIII digest of phage φX174 DNA, was included on each gel.

Restriction fragment length polymorphism analysis was conducted with at least one isolate from each tortoise to confirm conclusively that the isolates obtained from naturally and experimentally infected tortoises were M. agassizii (2). Twenty-microtiter samples of products from the amplification procedure described above were incubated with 10 to 20 U of the endonuclease AgeI (New England Biolabs, Inc., Beverly, Mass.), which cuts the M. agassizii amplification product at nt 613, and 5 μl of reaction buffer at 25°C for 1 h, and the products were electrophoresed as described above. The procedure resulted in products of 434 and 142 bp from M. agassizii-positive samples, and all mycoplasma isolates in the study were confirmed to be M. agassizii.

**ELISA.** Specific antibody to M. agassizii was determined by ELISA as described previously (30). Ninety-six-well microtiter plates (Maxisorp F96; Nunc, Kamstrup, Denmark) were coated with 50 μl of a whole-cell lysate of M. agassizii 723 at 10 μg/ml in phosphate-buffered saline (PBS) with 0.02% azide (PBS-AZ). The plates were incubated overnight at 4°C, washed four times with PBS-AZ plus 0.05% Tween 20 (PBST) in an automatic plate washer (EL403; Bio-Tek Instruments, Inc., Winooski, Vt.), and blocked overnight at 4°C with 250 μl of PBST containing 5% nonfat dry milk (PBS-TM) per well. Following washing, 50 μl of polyclonal antisera appropriately diluted from a specific study with anti-M. agassizii was added to each individual wells in duplicate or triplicate, and the plates were incubated at room temperature for 4 h in a humid chamber, and the plates were incubated for 60 min. Following washing, a conjugate of alkaline phosphatase and streptavidin (Zymed Laboratories, Inc., San Francisco, 0.5 ml (0.25 ml/nostral) of sterile SP4 broth. Monitoring and housing were identical to those in the initial experimental infection study.

**Postinfection monitoring of tortoises.** The tortoises were observed from 5 to 7 days after infection, including multiple objective measures of individual behavior patterns, not every tortoise was observed at each daily observation point. At specific time points (usually 2- to 4-week intervals, depending on the study), tortoises were captured by hand or with wire cage-type traps (Tomahawk Live Trap Company, Tomahawk, Wis.) that were covered with brown paper to protect the animals from the weather. The traps were cleaned, sprayed with bleach solution, and allowed to air dry following each use. The paper was discarded, and fresh paper was used for the next trapping effort. Each tortoise included in a plastic, labeled container (Lindgren Corporation, Watertown, Wis.) for transport and holding. Containers were bleached, scrubbed, and washed in an automatic cage washer before reuse. Tortoises were examined for clinical signs of URTD: nasal and ocular discharge, palpebral edema, and conjunctivitis. A photographic record consisting of right, left, and full face views was made for each tortoise at each time point when the tortoises were captured. The signs were graded individually on a scale of 0 to 3, which indicated none, minimal, mild, and severe signs, respectively. Visual grading of signs was confirmed by independent observation of the photographic record. Serum was obtained for quantitation of specific antibody. Nasal swabs and lavages were obtained for culture and PCR testing.
RESULTS

Clinical disease outcome: experimentally infected tortoises. M. agassizii caused clinical signs compatible with those observed in animals with natural infection (Table 1; see also Fig. 2). Clinical signs of URTD were evident in seven of nine experimentally infected tortoises by 4 weeks p.i. and in eight of nine experimentally infected tortoises by 8 weeks p.i. (Table 1).

The one tortoise which failed to show clinical signs did seroconvert, indicating that the animal had been colonized sufficiently to stimulate a host immune response. At all time points p.i., M. agassizii could be isolated from the nares of at least 50% of the tortoises (Table 1). After 8 weeks p.i., the ELISA was the most reliable method of detection, with 100% of the infected tortoises testing positive for antibodies to M. agassizii (Table 1). Control tortoises which were sham inoculated with sterile broth did not show clinical signs and did not seroconvert, and M. agassizii was not detected in these tortoises by either culture or PCR at any point in the study.

The cumulative scores for infected and control tortoises were different at all time points p.i. (P < 0.001) (Fig. 1A). Nasal discharge was statistically greater in infected tortoises than in control tortoises at 4 (P = 0.004), 8 (P = 0.01), 12 (P = 0.004), and 16 (P = 0.01) weeks p.i. (Fig. 1B). Ocular discharge was statistically greater in infected tortoises than in control tortoises at 4 (P = 0.01), 12 (P = 0.004), and 16 (P = 0.005) weeks p.i. (Fig. 1B). Palpebral edema was statistically greater in infected tortoises than in control tortoises at 4 (P = 0.04), 8 (P = 0.004), and 12 (P = 0.004) weeks p.i. (Fig. 1B). Conjunctivitis was statistically greater in infected tortoises than in control tortoises at 12 (P = 0.04) weeks p.i. (Fig. 1B).

The overall cumulative severity of clinical signs increased and then reached a relative plateau (Fig. 1A). However, the individual clinical signs comprising the cumulative scores showed significantly more variability (Fig. 1B). The severity of palpebral edema and conjunctivitis remained relatively constant throughout the 16-week observation period. The severity of nasal and, to a lesser extent, ocular discharge did increase with time following infection (Fig. 1B).

Considerable variability in the expression of clinical signs among individual animals occurred (data not shown). Some animals showed a classical plateau response, while others clearly demonstrated intermittent clinical signs. Some individual animals had very severe clinical signs, while others (n = 3) had clinical signs which had relatively low scores and one animal showed no clinical signs.

Infection with M. agassizii resulted in detectable antibody responses by week 8 p.i. (Fig. 2). All of the experimentally infected tortoises seroconverted. Levels of antibody were statistically higher in infected animals than in control animals for all time points >4 weeks p.i. (P < 0.0001) (Fig. 2). No antibody response was detected in any control animal at any time point.

Clinical disease outcome: dose-response study. The numbers of M. agassizii used to infect the tortoises initially did not influence the clinical expression of URTD. In most instances, the most severe clinical signs were observed at 8 weeks p.i., regardless of the infective dose. As was seen in the earlier infection trial, there was considerable variability in the expression of clinical signs by individual animals (data not shown). The antibody response (Fig. 3), like the expression of clinical signs, was not affected by the infection dose used. The antibody response in infected animals was first detectable at 6 weeks p.i. and was statistically different from that in the control animals at all time points thereafter (P < 0.001).

Histology: experimentally infected tortoises. The nasal cavities of control tortoises consisted of a dorsal multilayered olfactory epithelium (Fig. 4A) and a ventral mucous epithelium consisting of mucus cells intercalated with ciliated epithelial cells (Fig. 4B). Eight of nine experimentally infected tortoises had changes in the nasal epithelia and submucosa (Fig. 4B).
5). The epithelium was intact in all nine tortoises, and no ulcerations were present. One tortoise had mild to moderate changes, five tortoises had moderate changes, and three tortoises had changes that were characterized as moderate to severe.

**Histology:** dose-response study. Changes were observed in the nasal cavity epithelia of experimentally infected tortoises. Eight tortoises were selected for full necropsy. The changes...
observed, like the clinical signs, were variable and appeared to be independent of infective dose. In at least one tortoise, changes were different in the right versus left nasal cavity, suggesting that intra-animal variation also occurred. In the high-dose group ($n = 2$), one animal had mild to moderate inflammatory changes; the other tortoise showed moderate changes. Three tortoises in the medium-dose group were selected for necropsy. One tortoise that received the medium
The relative insensitivity of PCR versus the sensitivity of culture was somewhat surprising. Electron micrographic studies have identified preferentially colonized sites on the mucosal surfaces of ventrolateral depressions in tortoise upper respiratory passages, which may not be accessible by swabbing or lavage (data not shown). Since a prolonged incubation is required for culture, small numbers of *M. agassizii* may be expanded to a detectable level as a result of microbial growth. Despite its relative insensitivity, PCR still can play an important role in the diagnosis of URTD. A positive PCR result can be obtained with broth cultures after 24 to 48 h of growth, even though the initial PCR with the lavage specimen was negative. An additional problem encountered in diagnosis is the quality of samples. Since gopher tortoises are burrowing animals and many sample collections are done in the field under less than ideal conditions, the samples are often contaminated with bacteria and fungi. Many of these, especially fungi, rapidly overgrow the cultures or alter the medium beyond the pH range tolerated by mycoplasmas for growth. Culture in SP4 broth for 48 h before taking an aliquot for PCR enhances detection of viable mycoplasma, and contamination with other sources of DNA does not interfere with the PCR (data not shown). Isolation of *M. agassizii* from broth or agar can take up to 6 weeks. If broth cultures are grown for 24 to 48 h and then tested by PCR, we can detect positive culture samples faster, which may be of primary importance if animals are being quarantined or held prior to relocation as part of recommended health surveillance to prevent the spread of disease.

A wide variety of potential virulence factors have been suggested for mycoplasmas, including superantigen production, surface antigenic variation, and host immunomodulation (32). It is not uncommon to see a wide variety in the virulence of different strains of the same species within a specific host (7, 8, 17, 28). The strain selected for our studies was obtained from a very ill, naturally infected tortoise. This particular strain appears to be highly virulent, as evidenced by the fact that initial infective doses of only 10 CFU were capable of causing

**DISCUSSION**

The most stringent requirements for definitive proof of a causative relationship between an infectious agent and a disease is the fulfillment of the Henle-Koch-Evans postulates (10, 11). In a free-ranging, wild animal which is also legally protected, this is a daunting challenge. The *M. agassizii* isolate used in these studies was obtained from an animal with clinical disease. In the present experimental infection studies, this isolate was cultured in vitro and was inoculated into clinically healthy animals which were free of mycoplasma infection for a period of several months as determined by culture, PCR, and serology. The experimentally infected animals developed clinical signs of disease (eight of nine animals) and produced specific antibody against the infectious agent (nine of nine animals). Both the appearance of clinical disease and the seroconversion occurred within reasonable time periods relative to the time of inoculation of the infectious agent. The lesions observed in the animals with the experimental infection were similar to those observed both in desert tortoises with natural and experimental infections (4, 16) and in gopher tortoises with natural infections (unpublished data). *M. agassizii* was recovered from the experimentally infected animals at various times p.i. Thus, we have fulfilled the Henle-Evans-Koch’s postulates and conclude that *M. agassizii* causes URTD in the gopher tortoise.

In this study, the patterns of the experimental infections as well as of the natural infections of gopher tortoises that were observed are consistent with existing knowledge of mycoplasmal respiratory infections in other species. The variability observed in the clinical expression of disease among individual animals is common with other mycoplasmal respiratory infections (32). In most animals, respiratory mycoplasmosis is typified as a slowly progressing, chronic, and seemingly clinically silent infection which may be exacerbated by environmental factors, stress, or other microbial agents (5, 29, 32, 35). Most hosts have difficulty in eliminating the mycoplasma, even in the presence of a strong immune response. In fact, the host immune response is critical for the development of lesions (32). Although overt clinical signs may be inapparent, lesions can range from microscopic to gross, with eventual loss of the normal respiratory epithelium architecture (4, 5, 16, 32). The increased numbers of inflammatory cells, particularly in foci, and the lymphoid hyperplasia observed in the lesions of experimentally infected gopher tortoises are consistent with respiratory mycoplasmosis in other species, including the desert tortoise (16, 32).

The relative insensitivity of PCR versus the sensitivity of culture was somewhat surprising. Electron micrographic studies have identified preferentially colonized sites on the mucosal surfaces of ventrolateral depressions in tortoise upper respiratory passages, which may not be accessible by swabbing or lavage (data not shown). Since a prolonged incubation is required for culture, small numbers of *M. agassizii* may be expanded to a detectable level as a result of microbial growth. Despite its relative insensitivity, PCR still can play an important role in the diagnosis of URTD. A positive PCR result can be obtained with broth cultures after 24 to 48 h of growth, even though the initial PCR with the lavage specimen was negative. An additional problem encountered in diagnosis is the quality of samples. Since gopher tortoises are burrowing animals and many sample collections are done in the field under less than ideal conditions, the samples are often contaminated with bacteria and fungi. Many of these, especially fungi, rapidly overgrow the cultures or alter the medium beyond the pH range tolerated by mycoplasmas for growth. Culture in SP4 broth for 48 h before taking an aliquot for PCR enhances detection of viable mycoplasma, and contamination with other sources of DNA does not interfere with the PCR (data not shown). Isolation of *M. agassizii* from broth or agar can take up to 6 weeks. If broth cultures are grown for 24 to 48 h and then tested by PCR, we can detect positive culture samples faster, which may be of primary importance if animals are being quarantined or held prior to relocation as part of recommended health surveillance to prevent the spread of disease.

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**FIG. 5.** Representative moderate to severe changes observed in the upper respiratory tracts of experimentally infected gopher tortoises. The epithelium (E) is hyperplastic, and there are diffuse accumulations of mixed inflammatory cells (IC) in the lamina propria. Hematoxylin and eosin stain was used.
both clinical disease and severe lesions. We have preliminary evidence that other strains of *M. agassizii* do not cause overt clinical disease, even when relatively high infective doses are used. These observations of strain variability are similar to those observed for respiratory mycoplasmosis in rodents and poultry (7, 8, 28).

For the most part, any given mycoplasmal species has a relatively limited range of host specificity. Because *M. agassizii* has a limited temperature range for growth and does not grow at 35°C (3a), it is highly unlikely that it represents a threat to humans or other mammals. Conversely, evidence suggests that other chelonians (turtles and tortoises) may be susceptible to *M. agassizii* (15, 16, 19, 31). In the past several years, we have seen clinical cases of URTD in tortoises and turtles from zoological collections as well as private collections. It is not uncommon for these reptiles to be housed together, without quarantine or determination of health status. In at least one instance, confiscated star tortoises were sent to a zoo and were later found to have URTD. We have demonstrated the presence of *M. agassizii* in these animals by serology, culture, and PCR. Treatment with antibiotics alleviates clinical signs but does not eliminate the infection (14a). Appropriate quarantine, screening, and health surveillance of reptiles in collections will be needed to protect animals from URTD. This is particularly important when confiscated shipments of animals with unknown health histories are distributed to zoological settings. Both the gopher and desert tortoises have, to various extents, legal protection due to their decreasing populations. In many cases, the management tools used are relocation of animals to wildlife sanctuaries or other suitable habitats. Until recently, wildlife management decisions have not included considerations of infectious diseases and the possible impact of these diseases on population health. The full impact of relocation efforts involving ill animals is yet to be determined and will require long-term monitoring studies.

Research on the impact of mycoplasmosis on wildlife has been limited, but reports of recent disease outbreaks in different wildlife species are provoking interest in mycoplasmosis as a newly emerging (or at least newly recognized) disease threat for wildlife. The most publicized disease outbreak has been seen in *Mycoplasma gallisepticum* infection of house finches, goldfinches, and blue jays (21, 23, 27). In 1993 an epizootic of polyarthritis occurred in juvenile farmed crocodiles (*Crocodylus niloticus*) in Zimbabwe (18, 25). A mycoplasma was isolated from the joints and lungs of affected crocodiles, was determined to be a previously unrecognized species, and was named *Mycoplasma crocodyli* (18, 25). In 1995, a systemic infection of captive adult American alligators at a private facility in Florida was associated with a different species of mycoplasmal that had also been previously unrecognized. Unlike the outbreak in crocodiles, the disease in alligators was characterized by a very high mortality rate (>70%) and widespread dissemination of the infectious agent within the tissues of infected animals (3).

Infectious diseases are an ever present risk to wildlife, particularly during situations in which animals are removed from their natural habitat for captive breeding programs or during conditions of stress such as release into new habitats, translocation, ecosystem perturbation, and encroachment on their habitats by urbanization (13, 24, 26, 27). Infectious diseases, their implications for population health, and their impact on the success of conservation and management plans are rarely considered in management issues. URTD is an excellent example of the importance of wildlife diseases in population biology. Coupled with habitat destruction and environmental stress factors such as drought, this disease is believed to be a major factor in declines of desert tortoise populations in the Mojave Desert (1, 15, 16). Increasing awareness of the role of infectious diseases has resulted in inclusion of disease monitoring and assessment of population health in management decisions in both the desert and tortoise populations.

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