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Review

Mycoplasmosis and upper respiratory tract disease of tortoises: A review and update

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ABSTRACT

Tortoise mycoplasmosis is one of the most extensively characterized infectious diseases of chelonians. A 1989 outbreak of upper respiratory tract disease (URTD) in free-ranging Agassiz's desert tortoises (*Gopherus agassizii*) brought together an investigative team of researchers, diagnosticians, pathologists, immunologists and clinicians from multiple institutions and agencies. Electron microscopic studies of affected tortoises revealed a microorganism in close association with the nasal mucosa that subsequently was identified as a new species, *Mycoplasma agassizii*. Over the next 24 years, a second causative agent, *Mycoplasma testudineum*, was discovered, the geographic distribution and host range of tortoise mycoplasmosis were expanded, diagnostic tests were developed and refined for antibody and pathogen detection, transmission studies confirmed the pathogenicity of the original *M. agassizii* isolate, clinical (and subclinical) disease and laboratory abnormalities were characterized, many extrinsic and predisposing factors were found to play a role in morbidity and mortality associated with mycoplasmal infection, and social behavior was implicated in disease transmission.

The translation of scientific research into management decisions has sometimes led to undesirable outcomes, such as euthanasia of clinically healthy tortoises. In this article, we review and assess current research on tortoise mycoplasmosis, arguably the most important chronic infectious disease of wild and captive North American and European tortoises, and update the implications for management and conservation of tortoises in the wild.

Introduction

Respiratory infection of tortoises was first reported in California, USA, in the 1970s in confiscated Agassiz's desert tortoises (*Gopherus agassizii*) with nasal exudates (Fowler, 1980a), and in the UK in the 1980s in captive Greek (*Testudo graeca*) and Hermann's (*Testudo hermanni*) tortoises with rhinitis (Lawrence and Needham, 1985). Viruses (Jackson and Needham, 1983), *Mycoplasma* spp. (Fowler, 1980b; Lawrence and Needham, 1985) and *Pasteurella testudinis* (Snipes and Biberstein, 1982; Snipes et al., 1995) were hypothesized as possible causes.

In the 1980s, major declines in desert tortoise populations in the Mojave Desert of California, USA (Berry and Medica, 1995), and an associated upper respiratory tract disease (URTD; Jacobson et al., 1991), led to desert tortoises in the Mojave Desert north and west of the Colorado River being declared threatened (US Fish and Wildlife Service, 1990). A similar disease was seen in both captive (Beyer, 1993) and wild (McLaughlin, 1990; Beyer, 1993) gopher tortoises (*Gopherus polyphemus*) in Florida, USA. A microbial and pathological study (Jacobson et al., 1991) resulted in the identification of a new mycoplasma, *Mycoplasma agassizii* (Brown et al., 1995) and the confirmation of its causal relationship with URTD in desert (Brown et al., 1994) and gopher tortoises (Brown et al., 1999b).

Tortoise mycoplasmosis has since become one of the most extensively characterized infectious diseases of chelonians. Seminal research studies include: (1) a description of the anatomy and histology of the upper respiratory tract of healthy and affected tortoises (Jacobson et al., 1991); (2) identification and characterization of two new *Mycoplasma* spp. (Brown et al., 1995, 2001, 2004); (3) fulfillment of Koch's postulates, establishing that *M. agassizii* is a causative agent of URTD (Brown et al., 1994, 1999b); (4) development (Schumacher et al., 1993) and refinement (Wendland et al., 2007)

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of an ELISA to determine exposure of tortoises to *M. agassizii* (Brown et al., 1999a and b); (5) development of a conventional PCR (Brown et al., 1995, 2004) and a quantitative PCR (qPCR; DuPré et al., 2011) to detect *M. agassizii* and *Mycoplasma testudineum* DNA; and (6) correlation of specific antibodies against *M. agassizii* and *M. testudineum* with upper respiratory tract lesions in infected tortoises (Homer et al., 1998; McLaughlin et al., 2000; Jacobson and Berry, 2012).

In this article, we review these (and other) key studies and assess new research to update the current state of knowledge on mycoplasmal URTD in tortoises and its implications for management and conservation of tortoises in the wild.

Species of *Mycoplasma* in tortoises

Two mycoplasmas have been isolated from desert and gopher tortoises, and characterized: *M. agassizii*, originally isolated from a desert tortoise with URTD (2001), and *M. testudineum*, a genetically distinct organism (Brown et al., 2004). Both organisms cause similar lesions in the nasal cavities of tortoises, with those caused by *M. testudineum* possibly being less severe (Jacobson and Berry, 2012). A third mycoplasma, *Mycoplasma testudinis*, was isolated from the cloaca of a healthy pet Greek tortoise in England (Hill, 1985) and has not been associated with URTD. Recently, a novel, unnamed, *Mycoplasma* sp. was identified by genomic sequencing in a sample obtained from the phallus of a wild desert tortoise (Wellehan et al., 2014).

Hosts and geographic distribution of mycoplasmas of tortoises

Evidence of infection with *M. agassizii* in many species of wild and captive tortoises across the world has been determined using serology, PCR and/or culture. Most information for wild tortoises pertains to gopher tortoises (Beyer, 1993; Berish et al., 2000, 2010; McLaughlin et al., 2000; Wendland, 2007) in South-Eastern USA, both Agassiz's (Jacobson et al., 1991, 1995; Lederle et al., 1997; Christopher et al., 2003; Dickinson et al., 2005; Johnson et al., 2006) and Morafka's (*Gopherus morafkai*, formerly *G. agassizii*; Dickinson et al., 2005; Jones, 2008; Murphy et al., 2011) desert tortoises in South-Western USA, and the Texas tortoise (*Gopherus berlanderi*; Guthrie et al., 2013) in Texas, USA.

In Europe, mycoplasmas have been identified in wild spur-thighed tortoises (*Testudo graeca graeca*) in Morocco, wild Hermann's tortoises in France (Mathes et al., 2001; Mathes, 2003), captive Hermann's and spur-thighed tortoises in France (Mathes et al., 2001; Mathes, 2003), wild spur-thighed, Hermann's and marginated (*Testudo marginata*) tortoises in Italy (Lecis et al., 2011), captive spur-thighed and Russian (*Testudo*, formerly *Agrionemys*, *horsfieldii*) tortoises in Spain (Salinas et al., 2011), and captive spur-thighed, Hermann's, Russian and leopard (*Stigmochelys*, formerly *Geochelone*, *pardalis*) tortoises in the UK (McArthur et al., 2002; Soares et al., 2004). Mycoplasmas have also been identified in many captive non-native pet tortoises in the USA (Brown et al., 2002; Wendland et al., 2006).

M. testudineum was originally isolated from the nasal cavity of a clinically ill desert tortoise from the Mojave Desert, USA (Brown et al., 2004). This organism was subsequently identified in three wild gopher tortoise populations in North-Eastern Florida (Wendland, 2007).

Mycoplasma spp. also have been identified in other chelonians, including free-ranging Eastern box turtles (*Terrapene carolina carolina*) with URTD in Virginia, USA (Feldman et al., 2006) and a captive ornate box turtle (*Terrapene ornata ornata*) in Hungary (Farkas and Gál, 2009). *M. agassizii* has also been identified by PCR in the lungs of red-eared sliders (*Trachemys scripta elegans*) with pneumonia from Louisiana, USA (J. Roberts and E. Jacobson, unpublished data).

Clinical disease and pathology

Clinical vs. subclinical infection

Clinical signs of mycoplasmosis in tortoises include palpebral edema, conjunctivitis, and nasal and ocular discharges (Jacobson et al., 1991; McLaughlin et al., 2000; Mathes, 2003; Jacobson and Berry, 2012). However, subclinical infection with *Mycoplasma* spp. also occurs (Jacobson et al., 1995). Cycles of convalescence and recrudescence of clinical signs have been observed in captive and free-ranging desert and gopher tortoises (Brown et al., 1999a and b).

Histopathology

Mycoplasmosis in tortoises is typically seen as an URTD, primarily affecting the nasal cavity (Jacobson et al., 1991, 1995). Pneumonia is occasionally seen. Histologically, normal nasal cavities of tortoises consist of a ventral, mucous and ciliated, epithelial mucosa, and a dorsal, multilayered, olfactory epithelium. In tortoises with mycoplasmosis due to *M. agassizii*, lesions in the nasal cavity may be focal to diffuse, minimal to severe and may include basal cell hyperplasia in the mucosa, infiltrates of heterophils and histiocytes, and lymphoid hyperplasia in the submucosa. Depending on the epithelial changes and the extent of the inflammatory response, the following categories have been used to classify lesions: (1) mild inflammation; (2) moderate inflammation, and (3) severe inflammation (Jacobson et al., 1995).

In a group of desert tortoises that were serologically positive for *M. testudineum*, lesions in the nasal cavities were less diffuse and severe than in desert tortoises infected with *M. agassizii* (Jacobson and Berry, 2012). This could indicate that *M. testudineum* is less pathogenic than *M. agassizii*, or that the desert tortoises were more recently infected.

Serology

Serological assays

An ELISA was developed to detect antibodies against *M. agassizii* in plasma and serum using a monoclonal antibody (MAb HL673) against the light chain of desert tortoise immunoglobulins IgY and IgM (Schumacher et al., 1993). The antigen used in the ELISA was derived from *M. agassizii* PS6, the type strain from a desert tortoise with URTD. The reactivity of MAb HL673 was validated by Western blot analysis (Schumacher et al., 1993) and reference polyclonal *T. horsfieldii* IgY and IgM antisera were obtained from H. Ambrosius, Leipzig, Germany (Ambrosius, 1976). The Mab HL673-based ELISA was further validated using experimental transmission studies in desert (Brown et al., 1994) and gopher tortoises (Brown et al., 1999b). In these studies, reference standards that were independent of the mycoplasmal diagnostic tests were presence of clinical signs and histological lesions (Schumacher et al., 1997; Brown et al., 2002).

An ELISA was also developed to determine exposure of gopher and desert tortoises to *M. testudineum* using *M. testudineum* CB57 as the antigen (Jacobson and Berry, 2012). In studies with >1000 tortoises (M. Brown, unpublished data), relatively few serum samples reacted with both *M. agassizii* and *M. testudineum*, and those samples that reacted with both *Mycoplasma* spp. were from tortoises in populations with documented presence of both pathogens. As new species of mycoplasmas are isolated from tortoises, validation and standardization of serological assays will be required.

Whereas the original ELISA results were reported as an enzyme immunoassay (EIA) ratio (Schumacher et al., 1993), the reporting system was eventually converted to end-point titers (Wendland et al., 2007). Results for ~6000 independent desert and gopher tortoises