Management of *Mycoplasma agassizii* infection in a Horsfield tortoise (*Testudo horsfieldii*)

A 3-year-old Horsfield tortoise was presented for lethargy, anorexia, blepharedema and mucopurulent ocular and nasal discharge. Culture of nasal exudates was negative, but real-time polymerase chain reaction on an oral swab revealed the presence of *Mycoplasma agassizii*. Multimodal therapy consisted of ocular and nasal flushing, systemic and topical antibiotics, nebulisation, analgesia and supportive care in the form of warmth, fluid therapy and nutritional support. As *Mycoplasma* spp. remain latent within the body, correction of husbandry and nutritional deficits was particularly important to enhance the animal's immune system, in order to prevent recrudescence of clinical signs. To avoid disease spread, the client was advised against introducing new tortoises into the collection.

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Mycoplasma agassizii is a well-known respiratory pathogen found in captive and wild chelonians, and infection can lead to significant morbidity and mortality. The objective of this clinical case report is to educate veterinary practitioners on superior diagnostics and therapeutics used for *M. agassizii* infection, to emphasise the importance of a multimodal approach to diagnostics and therapeutics when dealing with upper respiratory disease in tortoises, and to create awareness of the important role of veterinarians in the prevention of transmission and recrudescence of clinical signs by means of client education.

Signalment

A 3-year-old male Horsfield tortoise (*Testudo horsfieldii*), weighing 0.085kg.

History

The patient was presented because of a 7-day history of inappetence, lethargy, blepharedema and bubbling from the nares. It had recently been rescued from living in suboptimal housing conditions and was then introduced to a female Horsfield's tortoise in the client's greenhouse. No previous health concerns had been communicated to the new owner.

The diet consisted of dandelions, cucumber and tomato; calcium was supplemented by means of a chalk block. The greenhouse contained a grass substrate and had a thermostat-controlled incandescent light bulb. Hiding spots, interest and sleeping quarters were available and free access to the outdoors was provided to allow the tortoises to reach cooler temperatures.

Physical examination

The tortoise was dull in demeanour and in poor body condition. Slight atrophy of the temporalis muscle and a visible sagittal crest suggested a body condition score of 3/9, based on the body condition scoring for desert tortoises (Lamberski et al, 2012).

There was bilateral palpebral oedema and the eyelids were sealed shut with a dry caseous substance. A 24-gauge catheter was inserted between the eyelids and the cornea and both eyes were flushed with sterile saline (0.9% sodium chloride, Vetivex I, Dechra, 1ml for each eye) to improve visualisation of the (peri) ocular structures. Moderate chemosis and conjunctival hyperaemia covered a substantial part of the sunken eyes, so hindered ophthalmic examination. There was bilateral mucopurulent nasal discharge without erosion of the nares, wheezing or increase in

respiratory effort. Oral inspection, Doppler cardiac auscultation and assessment of the pre-femoral fossae and cervicobrachial windows revealed no abnormalities.

Problem list and differential diagnoses

When chelonians present with bilateral blepharedema, conjunctivitis and rhinitis, an infectious nature is commonly suspected. While various infectious agents, including bacteria (primary or opportunistic pathogens), viruses, fungi or parasites, can be involved, the most common pathogens isolated are *Mycoplasma* spp., herpesviruses and ranaviruses. Other potential causes of blepharedema can be trauma or the presence of substrate behind the eyelids, corneal conditions, orbital abscesses, neoplasia or, less likely, hypovitaminosis A. Anorexia often evolves from illness caused by infection, pain, metabolic disturbances, poor husbandry or neoplasia, and in many cases is concurrent with lethargy.

Diagnostics

A plain oropharyngeal swab was submitted for a quarantine profile which involved Herpesvirus polymerase chain reaction (PCR) assay, *M. agassizii*-PCR, picornavirus-PCR, ranavirus-PCR and mycology, and the animal was found to be positive for *M. agassizii.* Culture and sensitivity performed on nasal exudates yielded negative results. Nasal exudates were obtained through a nasal flush, which involved infusion of isotonic saline into both nares with the animal being held in a diagonal position with its head towards the floor. Haematobiochemistry analysis performed on venous blood, obtained from the right jugular vein, showed a hyperuraemia (4.3 mmol/litre; 0.00–2.10mmol/litre), hypocalcaemia (iCa 0.85mmol/L; >2.0mmol/litre), leucopaenia (0.5 10^9/litre; 1.0–4.5), with toxic neutrophils on a blood smear and a mildly elevated packed cell volume (43%; 17–42).

Diagnosis

Upper respiratory tract infection caused by *M. agassizii* infection.

Therapeutics

The tortoise's poor state of health necessitated hospitalisation. Owing to the infectious nature of *M. agassizii*, the patient was isolated and strict barrier nursing was implemented. Treatment consisted of supportive care, analgesia, nasal flushing, topical and systemic antimicrobials. To improve hydration, the tortoise was bathed daily in lukewarm water supplemented with Reptoboost (Vetark, one scoop in 500ml of water). Furthermore, epicoelomic fluids (Ringer Lactate solution, Vetivex II, Defra; 35ml/kg/day on day 1, followed by 20ml/kg per day for 3 more days) were administered by injection through the cranial inlet of the shell, lateral to the head, dorsal to the plastron.

Analgesia consisted of tramadol (Tramadol, Zydol, 5mg/kg intramuscular, single dose), delivered during admission, which was replaced by meloxicam (Meloxidyl, Ceva, 0.5mg/kg per os every 48 hours for three doses), following fluid support 12 hours later. Once warmed and re-hydrated, nutritional support (Emeraid Herbivore, Lafeber, 1ml/100g per os daily) was given by means of a gavage tube placed into the stomach.

Systemic antibiotic therapy consisted of doxycycline (Karidox, Nimrod, 10mg/kg per os every 24 hours for 7 days) and this was continued for 3 days based on the positive *M. agassizii* PCR. Topical antibiotics consisting of ciprofloxacin (0.3% Ciloxan, Alcon, topically every 12 hours for 7 days) in both eyes and over the nares were started, but discontinued following negative culture results. F10 antiseptic was used for twice-daily nebulisation and to clear nasal exudates by means of a nasal flush every 12 hours for 4 days. To counteract hypocalcaemia Zolcal-D (Vetark, 0.1ml/100g per os daily for 7 days, then once weekly for 14 days) was provided. Following the animal's discharge, the enclosure was disinfected with a 5% bleach solution to prevent environmental transmission of *M. agassizii* towards new hospitalised patients.

The client was educated that, once recovered from active infection, chelonians become chronic subclinical carriers where any environmental or medical cause of immunosuppression results in recrudescence. With the assumption of its cage mate being the potential source of infection, the tortoises were not separated, but its highly infectious nature urged the need for permanent isolation of both tortoises from naïve chelonians. With the view to optimise the tortoise's immune system, the client was advised to substitute grass with a 50/50 soil and sand mix as it can be a contributing factor to respiratory disease when damp. An educational handout on healthy tortoise foods, such as weeds (for example, chickweed) and garden plants (such as marigolds, was provided.

Outcome

Improvement in its demeanour, appetite and vision allowed the tortoise to be discharged 4 days after initiation of therapy. The decrease in palpebral oedema had allowed for ocular examination which revealed a normal globe bilaterally. Successful treatment was confirmed 7 days later during re-examination, which showed complete resolution of all clinical signs. One year later, during a pre-hibernation consultation, the tortoise appeared healthy with no evidence of respiratory disease.

Discussion

Mycoplasmosis has emerged as a complex multifactorial upper respiratory disease in wild and captive chelonians (Jacobson et al, 2014) and its spread across the world has been linked to human actions (Stanford et al, 2020). Of 1015 captive chelonians in Europe, 42% have had active *Mycoplasma* spp. infection (Kolesnik et al, 2017). Horsfield tortoises appear more susceptible to *M. agassizii* than other commonly kept species, with 78% of tested captive Horsfield tortoises in the UK being positive (Soares et al, 2004). *M. agassizii* is recognised as one of the causative agents of the upper respiratory tract disease complex in chelonia (Chitty and Raftery, 2013).

Approach to diagnostics

Correlation of clinical signs, consisting of mucopurulent ocular and nasal discharge, palpebral oedema and conjunctivitis, raised the suspicion of respiratory infection (Jacobson et al, 2014), and made neoplasms and corneal conditions as causes of palpebral oedema (Chitty and Raftery, 2013) less likely. The latter was further ruled out by ocular examination once palpebral oedema had diminished. The absence of exophthalmos eliminated orbital abscesses as a dif-

ferential for palpebral oedema (Chitty and Raftery, 2013).

Mycoplasma spp. infection often can be clinically indistinguishable from infection with herpesvirus or ranavirus (Adamovisz et al, 2020), so it was important to test for all in order to establish a definitive diagnosis. Co-infection appears to be a common finding, so detection of one pathogen does not necessarily eliminate the presence of other organisms (Adamovisz et al, 2020). Although not ruled out by means of measuring serum vitamin A levels, hypovitaminosis A appeared less likely because chelonians are herbivores thus are able to convert dietary carotenoids into vitamin A (Boyer, 2019). Additionally, serum vitamin A levels only drop when liver levels are exhausted, so these are not easily measured (Boyer, 2019).

Real-time PCR is the favoured method for detection of *M. agassizii* and the positive result was immediate proof of active infection at the time of sampling (Jacobson et al, 2014). In a study on captive desert tortoises, PCR allowed detection of *M. agassizii* in 100% of the included animals (DuPré et al, 2011). Negative herpesvirus PCR results allowed for a better prognosis as often these pathogens may work synergistically, resulting in more severe clinical signs (Jacobson et al, 2014). Despite the risk of it providing false negatives, the oral swab remained the most suitable sample for detection of this pathogen because of its minimally invasive nature and higher sensitivity than nasal washes (Kolesnik et al, 2017); however, the latter could be used in difficult patients unwilling to open their mouths. For future cases, the author would now consider submission of both in order to increase detection rates (Kolesnik et al, 2017).

Culture of *Mycoplasma* spp. can be unrewarding because these pathogens often fail to grow in culture media (Chitty and Raftery, 2013). However, it was essential to identify opportunistic pathogens which often invade once mycoplasmosis has resulted in immunosuppression (Salinas et al, 2011). For future patients, the author would now consider in-house cytology, to include Gram-stain of nasal exudates, where the morphology and staining characteristics of certain bacteria may suggest their identity and antibiotic choice can be directed appropriately (Chitty, 2019). As such, in this case, topical antibiotic treatment may have not been started.

Although it was unlikely to provide more information on the respiratory disorder itself, blood analysis was fundamental for two reasons. First, it eliminated the presence of underlying medical factors of immunosuppression (Music and Strunk, 2016). Second, it demonstrated the extent of immunosuppression, which is particularly important for the management of mycoplasmosis as clinical disease often appears as a secondary complication to a compromised immune status (Chitty, 2019). With elimination of systemic disease and recognition of leucopaenia and hypocalcaemia as reflections of poor housing and nutrition (Rendle and Calvert, 2019) in this patient, environmental stress associated with the previous suboptimal conditions was thought to be the main contributing factor to the development of clinical mycoplasmosis.

Radiography was considered to be of no value because the fine structures in the upper respiratory tract and the lack of a well-defined sinus system make radiography for the diagnosis of upper respiratory tract disease insensitive (Chitty, 2019). However, as *M. agassizii* has the potential to cause lower respiratory tract infections (Studer and Girolamo, 2021), the author would now always include this method in their diagnostic approach.

Approach to therapeutics

Successful treatment of clinical mycoplasmosis was based on biological support and antibiotic therapy (topical and systemic) (Chitty, 2019). Besides warmth, rehydration and oral nutrition, Reptoboost (Vetark) may prove useful to provide debilitated animals with energy, electrolytes and probiotics. Vetark's Zolcal-D, an oral calcium and vitamin D3 supplement, is ideal in situations where rapid increase in calcium concentrations is desired (Holliday, 2014). Having reflected on this case, the author would now always repeat biochemical analysis to re-evaluate the patient's calcium status following treatment.

In the literature, scientific publications mostly focus on the incidence of mycoplasmosis in captive (Soares et al, 2004; Kolesnik et al, 2017) and wild collections (Berry et al, 2015), the subsequent implications for wild chelonian populations (Stanford et al, 2020), and comparison of the different methods (PCR, serology, culture) used for its detection (Kolesnik et al, 2017). However, case studies detailing its management in a veterinary setting are lacking. Fluoroquinolones, tetracyclines and macrolides (specifically clarithromycin and tulathromycin) have all been suggested to be useful to reduce clinical signs of *Mycoplasma* spp. infection (Hedley et al, 2021). When *Mycoplasma* spp. infection is suspected and systemic antibiotic treatment must be started while awaiting culture results, tetracyclines remain the first-line antibiotic of choice (Hedley et al, 2021). Determination of dosages, in this case, was based on recommendations in the exotic animal formulary (Carpenter, 2017). In reptile medicine, fluoroquinolones and third- and fourth-generation cephalosporins appear to be commonly used antibiotics (Hedley et al, 2021). Concerningly, both are classified by the World Health Organization (2018) as one of the groups of 'highest priority critically important antimicrobials' for human medicine, so these should not be used as a first-line antibiotic. In contrast to doxycycline depot injections, the oral form has the advantage of penetrating locally (Chitty, 2019). However, in cases where severe infection is observed, or administration of oral medication proves difficult, parenteral doxycycline at a dose of 50mg/kg intramuscularly every 7 days (Chitty, 2019) could be an option for countries in which this product is available.

Local treatment by nebulisation of antiseptics or antimicrobial medication with the aim to clear mucus, humidify the airways and acquire a high drug dosage at the area of pathology has been proposed (Chitty and Raftery, 2013), although there remains some controversy surrounding its efficacy, so it was used as an adjunct to topical antibiotic therapy (Chitty and Raftery, 2013).

Control

Chelonians are unable to eliminate infection (Wendland and Brown, 2019) and therefore become subclinical carriers. Client education on quarantine measures is not only vital to prevent disease spread within captive species (Marschang and Chitty, 2019), but also from a conservation point of view. This is because infected captive tortoises may pose significant risks to healthy naïve wild

populations if they were to ever escape or be intentionally released (Berry et al, 2015).

Because transmission rates are directly related to overt clinical signs (Jacobson et al, 2014), disease transmission was assumed to have occurred following direct contact (Berry et al, 2015) with the patient's cage mate. Serology on blood collected from its cage mate to detect antibodies would have been useful to confirm subclinical disease in this tortoise (Pasmans et al, 2008; van Zanten and Simpson, 2021).

The main difficulty lays with prevention of recrudescence, which may occur when factors such as chronic stress and poor nutrition cause immunosuppression (Aiello et al, 2018). Therefore, a comprehensive discussion on nutritional and environmental enrichment was an essential part of treatment (Chitty, 2019).

Conclusions

This case highlights the important role of the veterinary practitioner in the prevention of transmission and recrudescence of *M. agassizii* in a captive chelonian collection. The high incidence of mycoplasmosis in captive tortoises, whose wild counterparts are subject to habitat destruction and human predation, may lead to major declines in captive colonies which could be problematic should the species ever become endangered. CA

Conflicts of interest

The author declares that there are no conflicts of interest.

KEY POINTS

- *Mycoplasma agassizii* is a pathogen highly prevalent in captive chelonian collections with Horsfield tortoises being most susceptible.
- Real-time polymerase chain reaction testing is currently the favoured method for detection of *M. agassizii* in chelonians.
- Doxycycline is the current antimicrobial of choice to treat clinical mycoplasmosis.
- Appropriate nutritional and environmental care is essential to prevent recrudescence of clinical symptoms.
- Strict isolation of *Mycoplasma*-positive tortoises is necessary to control disease spread.

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