**ANTIBIOTIC STEWARDSHIP FOR REPTILES**

ABSTRACT

This review discusses the general principles underlying responsible antibiotic usage in reptiles. Very little evidence underlies antibiotic usage in reptiles, and there are no published guidelines for responsible antibiotic usage. A literature search was performed to review the evidence for bacterial involvement in the pathology of selected common diseases of reptiles, allowing development of recommendations for responsible antibiotic treatment of those diseases.

INTRODUCTION

There are increasing concerns regarding the impact of antimicrobial use on the emergence of antimicrobial resistance in both medicine and veterinary medicine (Gould, 2009; Bengtsson and Greko, 2014; Weese *et al.*, 2015). Meanwhile the development of new antibiotics has slowed, leaving both doctors and veterinarians with a finite number of options for treating microbial infections (Guardabassi *et al.*, 2004; WHO, 2017a). There has been a high clinical use of quinolones in both animals and humans in the last decade, with a study on prevalence and characterisation of quinolone resistance genes implying that pet turtle-associated *Proteus* spp. should be considered a potential source for the dissemination of antibiotic resistance genes (Guan *et al.,* 2013; Pathirana *et al.,* 2018).

Antimicrobial resistance has been reported in a variety of both free-ranging and captive reptiles (Cushing *et al*., 2011; Kim, Lee and Kwak, 2015; Unger *et al.*, 2017; Tang *et al.*, 2020). Antibiotic use in reptiles is a particular area for concern as only two antibiotics are licensed for reptiles in the UK; a fluoroquinolone and a potentiated sulphonamide that has only been licensed in bearded dragons for treatment of protozoal infections (**Table 1**).

**TABLE 1 – POM-V ANTIBIOTICS CURRENTLY LICENSED FOR REPTILES** (as listed on Veterinary Medicines Directorate (VMD) Product Information Database)

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| --- | --- | --- | --- |
| **Drug** | **Generic name** | **Datasheet dosages** | **Comments** |
| Enrofloxacin | Baytril; Elanco UK AH Limited, Enrobactin; Virbac, Enrocare; Animalcare, Enrotron; Forte Healthcare Limited | 5 mg / kg q24-48h | Oral and injectable formulations licensed for use in reptiles for the treatment of bacterial infections of the alimentary and respiratory tracts where clinical experience, supported where possible by sensitivity testing of the causal organism, indicates enrofloxacin as the drug of choice.  Higher doses are recommended in formularies at 5–10 mg/kg PO, IM q24-48h (Carpenter, 2018; Hedley, 2020) |
| Trimethoprim +  Sulfamethoxazole | Sulfatrim Oral Drops; Virbac Limited | 15 – 20mg/kg PO q24h (Noah compendium) | Only licensed in bearded dragons for treatment of gastrointestinal infections caused by protozoa |

Currently in the UK, when selecting any medication for an animal, VMD guidelines state that where an authorised product exists for treatment of that condition in that species, that product should be used first. Therefore, enrofloxacin would theoretically be the first choice for treatment of a bacterial infection in a reptile. However, the VMD has also published guidelines for responsible antibiotic use under the cascade, which state “it is justified, on a case-by-case basis, to prescribe an antibiotic on the cascade in the interests of minimising the development of resistance” (Veterinary Medicines Directorate, 2014). Consequently, the use of an alternative antibiotic may be justified, rather than first-line use of a licensed fluoroquinolone, if the veterinary surgeon feels that this is appropriate for the individual case and has informed owner consent.

Despite this, both fluoroquinolones and third- generation cephalosporins appear to be some of the most commonly used antibiotics in reptile medicine (Isaza and Jacobson, 2013; Hernandez-Divers *et al.*, 2017) . This is concerning as both fluoroquinolones and third- and fourth-generation cephalosporins are classified by the World Health Organization as one of the groups of “Highest Priority Critically Important Antimicrobials” for human medicine (WHO, 2017b).

BSAVA PROTECT ME Guidelines also state “In all species fluoroquinolones and third- and fourth-generation cephalosporins should be used judiciously. This means that their use as first-line agent should be avoided and they should only be used when other agents are ineffective (ideally determined by culture and sensitivity testing)”(BSAVA, 2019) . There is therefore obviously a need to assess, both why these antibiotic groups are used so commonly and whether alternative antimicrobials are feasible for use in reptiles.

An initial round-table discussion at BVZS Conference Autumn 2015 highlighted the following reasons for use of these drugs;

* Enrofloxacin is one of only two licensed antibiotics in reptiles in UK
* Lack of knowledge of alternatives
* Easily obtainable in small animal practice
* Easy to give at practical dosing intervals
* Clinically appear effective
* Side-effects appear uncommon
* Some pharmacokinetic evidence that appropriate concentrations in the blood can be achieved in selected species

The aim of this report was to examine published literature to identify the most common bacterial infections found in reptiles and provide, where known, evidence-based recommendations for antibiotic usage.

An electronic database search of Medline (Pubmed) and CAB Abstracts was performed by one of the authors in March 2021 to identify relevant literature. The following textbooks were also consulted (Girling and Raiti 2014, Divers and Stahl 2019). The keywords searched were “reptile”, first combined with “antibiotic”, then combined with “antimicrobial” and then with each of the clinical presentations discussed; “ocular”, “aural”, “stomatitis”, “periodontal”, “respiratory”, “dermatitis”, “shell disease” and “septicemia”. When research evidence is not referenced, proposals are given on critical, parsimonious theory and current knowledge. These recommendations do not replace a full diagnostic investigation including bacteriological and fungal culture and sensitivity testing.

Based on the literature search, there is only two bacterial diseases of reptiles for which Koch’s postulates have been fulfilled, definitively implicating bacteria as a primary cause of a disease: *Mycoplasma agassizii* and *M. testudineum* can cause upper respiratory tract disease (URTD) in some chelonian species (Brown *et al* 1999, Feldman *et al* 2006, Jacobson *et al* 2014), as can other infectious agents; and *Mycoplasma alligatoris* can cause fulminant inflammatory disease and rapid death in American alligators (Brown *et al* 2001) It is also important to note that the majority of bacterial infections in reptiles occur secondary to factors such as poor husbandry and immunosuppression or other infections including viral or fungal pathogens. Antibiotic treatment should only be prescribed when bacterial disease is present, and the primary problem should always be identified and resolved. Antibiotic treatment of the lethargic or anorexic reptile without a specific underlying problem having been identified is not indicated and may result in adverse side effects, in addition to potentially delaying diagnosis.

Antibiotic recommendations may be divided into those drugs that are suggested to be used first-line and those that should be reserved for second-line use (Weese, 2006; Divers *et al.*, 2017; Perry and Mitchell, 2019; WHO, 2017b). First-line drugs are appropriate antibiotics to use while awaiting or in the absence of culture and sensitivity results. Second-line drugs should ideally only be used if culture and sensitivity results indicate that first-line options are likely to be ineffective (**Table 2**). Third-line drugs include restricted or prohibited antibiotics that are reserved for multidrug resistant infections in human medicine. These are not to be used in veterinary medicine (Perry and Mitchell, 2019).

**TABLE 2 – FIRST- AND SECOND-LINE ANTIBIOTIC OPTIONS**

|  |
| --- |
| **First-line drugs**  To be used in the absence of or while awaiting results of culture and sensitivity testing, to prevent infection in severely compromised animals (persistently low white blood cell count) or peri-operatively for orthopaedic procedures   * Penicillins: penicillin G, amoxicillin, or ampicillin * Potentiated penicillins: amoxicillin-clavulanic acid * First-generation cephalosporins: cefazolin or cephalexin * Second-generation cephalosporins: cefoxitin, defuroxime (Zinacef®) * Trimethoprim-sulfonamide * Tetracyclines * Lincosamides * Metronidazole * Aminoglycosides   **Second-line drugs**  To be used if bacteriologic culture and antimicrobial susceptibility testing indicate that first-line options are likely to be ineffective.   * Advanced penicillins; Piperacillin, Ticarcillin * Fluoroquinolones * 3rd or 4th generation cephalosporins: ceftazidime (Fortum®), ceftiofur (Excenel®), cefotaxime * Macrolides |

It is important to note that there are few pharmacokinetic and pharmacodynamic studies to support the use of these drugs in the majority of reptile species. Drug doses are usually anecdotal and often extrapolated from doses used in other animals. Drug metabolism may also vary with body temperature, site of administration, season and health or disease state. Measuring antibiotic levels is an important consideration for assessing likely efficacy if possible. If in any doubt, especially when using a drug for the first time in a species, an up-to-date exotics formulary such as BSAVA Small Animal Formulary: Part B: Exotic Pets or Carpenter’s Exotic Animal Formulary and Mader’s Reptile and Amphibian Medicine and Surgery 3rd Edition should be consulted to ensure that no species-specific reactions have been reported.

There are no controlled trials demonstrating that antibiotic usage improves outcome for any reptile disease. Clinical experience indicates that antibiotic treatment is beneficial for many diseases, but clinical experience is notoriously unreliable in determining whether treatments are genuinely efficacious. Numerous case reports have been published in which reptiles with various diseases have been treated with antibiotics, in some of which the animals’ condition improved or resolved. However, even in reports in which antibiotic treatment was associated with improvement in a disease, it is often not clear that the antibiotic treatment itself led to the improvement, because of one or more of the following factors:

* No attempt made to determine whether bacteria were present.
* Presence of bacteria was confirmed, but no cytology or histopathology was carried out to support involvement of the bacteria in the pathology of the lesion.
* The bacterial species present were not identified, or even categorised to the level of Gram +/- and aerobic/anaerobic, so it was not known whether the antibiotic used would likely have been effective against those bacteria.
* It was clear in some reports that other – non-bacterial – pathological processes were involved in the disease, and in many reports, even where other pathological processes were obviously apparent, little or no attempt was made to rule out the presence of other aetiologies, including other infectious agents. These other pathological processes or causative agents may have been responsible for the disease, and the identified bacteria largely or completely incidental.
* In reptiles, sub-optimal husbandry – leading to immune-suppression, among other things – is a major aetiological component of bacterial disease in reptiles. Thus, changes in management were often implemented alongside antibiotic treatment, and may have been responsible for the animals’ improvement.
* Other medical or surgical treatments – targeted at known or suspected other aetiologies of the disease under treatment – were used alongside the antibiotic and may have been responsible for the animals’ improvement.
* Often, the disease was secondary to another bacterial disease, e.g., conjunctivitis thought to be secondary to stomatitis or septicaemia. In such cases, antibiotics may have improved or cured the primary disease, and the secondary disease also resolved, but it is not clear that this constitutes evidence that the secondary disease itself required antibiotic treatment – or would require antibiotic treatment in cases where it is the sole disease present.
* In some reports, two antibiotics were used, e.g., a topical and a systemic treatment, and it is not clear which, if either, led to the animals’ improvement.

Below, for each disease reviewed, we attempt to determine:

* The normal bacterial flora at the anatomical site.
* The bacteria associated with the disease at that site.
* Whether the disease has a significant bacterial pathology, and so whether antibiotic treatment is required or not.
* Whether the bacteria cultured from the disease, or identified by PCR, contribute substantially to the pathology.
* If antibiotic treatment is required:
  + Which antibiotics should be chosen empirically, for first-line use.
  + Whether culture or PCR techniques for identifying the species of bacteria present, and determining antibiotic-sensitivity profiles for isolated bacteria, are likely to be of benefit in improving antibiotic selection.

**TYPES OF PRESENTATIONS**

**OCULAR INFECTIONS**

Ocular infections in reptiles may vary from mild conjunctivitis to severe blepharitis. In mild cases topical medications can be used however, in the more severe cases, the use of systemic antibiotics might be warranted, and good antibiotic stewardship needs to be considered. Infections are usually due to the overgrowth of secondary opportunistic bacteria and secondary to primary causes such as foreign bodies, trauma, neoplasia or underlying systemic disease. Underlying husbandry or nutritional deficits may also play a role such as poor water quality for aquatic species and hypovitaminosis A. Mucopurulent ocular discharge is not a typical finding in bacterial conjunctivitis in reptiles due to the heterophils lacking lysosomes and instead, conjunctival plaque formation may occur ( Smith Fleming, 2019).

Studies have been performed to establish the range of normal conjunctival bacteria in a few reptile species, with variable results. In iguanas and leopard geckos, a range of bacteria have been shown to be present, but Gram positives such as *Staphylococcus* species predominate ( Cordeiro de Araujo *et al.,* 2017; Camacho-Luna *et al.*2020).

In terrestrial chelonian species such as red-foot tortoises, Gram positive bacteria are also mainly found (Galosi *et al*. 2021), whereas in aquatic species, Gram negative bacteria are the predominant population including *Aeromonas hydrophila and Pseudomonas species* (Di Ianni *et al.*, 2015; Somma *et al.*, 2015; Musgrave *et al.,*2016)*.* In 20 captive Aldabran tortoises (*Aldabrachelys gigantea*) from two UK collections, 100% of 40 eyes yielded 92 cultures, with a wide range of Gram positive (70%) and Gram negative (30%) bacteria isolated. No anaerobes were isolated. *Enterococcus* spp., coagulase-negative *Staphylococcus* spp., non-lactose fermenting coliforms and *Micrococcus* spp. were the most commonly isolated, between them making up 57% of species isolated (Wissink-Argilaga 2016). In 10 captive Sulcata tortoises (*Centrochelys sulcata*) from one UK collection, 100% of 20 eyes yielded 53 cultures, with a wide range of Gram positive (40%) and Gram negative (60%) bacteria isolated. No anaerobes were isolated. *Acinetobacter* spp. (40%) and coagulase-negative *Staphylococcus* spp. (34%) were the most commonly isolated (Wissink-Argilaga 2016).

Bacterial isolates cultured from three lizards in a zoological collection include *Morganella* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp. and *Serratia* spp. (Williams *et al.,* 2000). All isolates cultured were susceptible *in vitro* to gentamicin and enrofloxacin, with variable susceptibilities to fusidic acid. The clinical response did not always follow *in vitro* sensitivity data. *Staphylococcus* spp., *Corynebacterium* and *Branhamella* spp. were isolated from two tortoises with bacterial conjunctivitis. All isolates were susceptible to cefuroxime and enrofloxacin, with variable susceptibility to fusidic acid (Williams *et al.,* 2000). In a study evaluating selected diagnostic tests in Green iguanas, it was suggested that polymyxin B was the least effective antibiotic for Gram-positive and Gram-negative conjunctival isolates, whereas most of the bacterial isolates were susceptible to fluoroquinolones and the aminoglycosides (Cordeiro de Araujo *et al.,* 2017).

Chloramphenicol has a broad spectrum of activity against Gram-positive, Gram-negative and obligate anaerobic bacteria, so is an appropriate first line topical antibiotic for the majority of chelonian and lizard superficial ocular infections. If ineffective, culture and sensitivity is recommended. Whilst awaiting results, cytology can be performed to determine whether cocci or rods predominate or whether there are any fungal infections involved. If rods predominate, a topical aminoglycoside such as gentamicin or fluoroquinolone such as ciprofloxacin would be recommended (Holmberg , 2008).

In snakes and certain lizard species, sub-spectacular infections are more common, often involving *Pseudomonas or Salmonella* (Hausmann *et al.*, 2013)*.* Other bacterial isolates include *Proteus* spp., *Providencia rettgeri* and *Staphylococcus* spp. and *Clostridium perfringens* (Millichamp *et al.,* 1983; Hausmann *et al.,* 2013; Cleymaet *et al.*2020) and *Serratia* spp. from culture and sensitivity data from one of the author’s cases. Infection usually originates from either penetrating trauma, ascending infection via the lacrimal duct from the mouth or haematogenous spread. Purulent material accumulates in the subspectacular space between the spectacle and cornea. A subspectacular wedge resection is usually required to remove purulent material in addition to flushing and ensuring patency of the nasolacrimal duct. Samples can be taken for culture at this time. If there are no concurrent systemic signs, antibiotic cover can be provided topically by infiltrating a topical aminoglycoside into the subspectacular space after drainage (Lawton, 2019). If concurrent stomatitis is present, this should be treated systemically (see **Oral infections**).

In chelonian species, ocular mycoplasmosis should also be considered as a common cause of bilateral blepharitis and conjunctivitis (Jacobson *et al.*2014). Ocular abnormalities are usually associated with respiratory signs and systemic treatment is usually required (see **Respiratory infections**).

Recently, a novel Chlamydia species has been associated with ocular pathology in captive spur-thigh tortoises (Laroucau *et al,* 2020)

**CHELONIAN AURAL INFECTIONS**

Abscessation of the middle ear cavity is common in some chelonian species. Aural abscesses are typically treated by surgical debridement and topical antiseptic or antimicrobial (Kischinovsky and Divers, 2019), often with systemic antibiotics. No studies have been done to determine whether systemic antibiotic treatment of aural abscesses improves outcome over surgical debridement plus topical antiseptic/antimicrobial treatment alone. Thus, despite their frequent use alongside surgical debridement of uncomplicated aural abscesses, it is not known whether systemic antimicrobials are of benefit in these cases. As reptilian abscesses contents have no blood supply, systemic antibiotics probably penetrate the caseous pus poorly if at all, and use of systemic antibiotics is not an alternative to surgical debridement.

Only one study (Joyner *et al* 2006) has investigated the bacterial flora of the normal chelonian middle ear, and in only one species, the Eastern box turtle (*Terrapene carolina carolina*), in which bacteria were isolated from 10 of 15 grossly normal aural cavities of free-living wild specimens. Typically, only one or two bacterial species were isolated per ear, being a range of Gram-positive and Gram-negative organisms, with no bacterial species consistently present across individual tortoises, suggesting an environmental origin of the bacteria – which presumably reached the middle ear via the Eustachian tubes.

It is not known how generalisable the reported bacterial isolates are to other chelonian species, to Eastern box turtles maintained in captivity, or even to free-living wild Eastern box turtles from other geographical locations. If the normal flora is primarily of environmental origin, they likely vary with environment depending on temperature, season and other factors.

Thus, the available evidence does not allow determination of whether or not there is a consistent ‘normal flora’ of the middle ear of any of the reptile species that have middle ear cavities.

Studies and case reports have isolated bacteria from aural abscesses of Eastern box turtles (Tangredi and Evans 1997, Willer *et al* 2003, Joyner *et al* 2006, Feldman *et al* 2006), and red-eared sliders (*Trachemys scripta elegans*) or other emydid turtle species (Jang and Biberstein 1991, Yardimci *et al* 2010, Acik *et al* 2018, Bae *et al* 2020).

In free-living Eastern box turtles, typically only one or two species were isolated per aural abscess, being a wide range of Gram-positive and Gram-negative organisms, with no bacterial species consistently present across individual tortoises, suggesting an environmental origin of the bacteria. There was no strong overlap of the bacterial species isolated from aural abscesses across the three reports (Tangredi and Evans 1997, Willer *et al* 2003, Joyner *et al* 2006). The most commonly isolated organisms were *Proteus* spp., *E. coli*, *Aeromonas hydrophila*, *Morganella morganii* and *Pseudomonas* spp., but together these species accounted for less than half of the isolates across these three studies.

*Mycoplasma agassizii* and *M. testudineum* cause upper respiratory tract disease (URTD) in multiple chelonian species, including Eastern box turtles (Brown *et al* 1999, Feldman *et al* 2006, Jacobson *et al* 2014). Feldman *et al* (2006) found no relationship between aural abscesses and *Mycoplasma* infection in wild Eastern box turtles with and without URTD.

Despite histopathology indicating the presence of large colonies of heterogenous gram-positive and gram-negative bacteria (Brown *et al* 2004), only one or two bacterial species were isolated per Eastern box turtle aural abscess. Further, the culture methods in the published studies were almost entirely aerobic, so although anaerobic bacteria were not found they may have been present. These considerations suggest that isolated bacterial species were only a subset – possibly a small subset – of the bacteria present in aural abscesses.

A wide range of bacteria have also been isolated from aural abscesses of emydid turtles. These bacteria overlap with those reported in Eastern box turtles, but there are differences. In particular, the chelonian commensal *Pasteurella testudinis* appears commonly associated with aural abscesses of the aquatic turtles, but not of Eastern box turtles or other tortoises (Jang and Biberstein 1991, Yardimci *et al* 2010, Acik *et al* 2018, Bae *et al* 2020). Thus, it is not necessarily the case that the bacteria isolated from aural abscesses of one chelonian species are representative of those in other chelonian species.

Even though some of the bacteria isolated from aural abscesses are potential pathogens, it is not clear which, if any, of those isolated in any of the published reports were contributors to the pathology of the abscess – they may have been secondary contaminants, or incidental commensals well suited to growing in the conditions of the diseased middle-ear cavity. In particular, although *P. testudinis* has been found in associations with some chelonian diseases (Jang and Biberstein 1991, Henton 2003), no studies have investigated whether, in aural abscesses, it is a primary pathogen, a secondary pathogen among a larger flora, or an incidental commensal.

These considerations imply that there may be little benefit in bacterial culture and sensitivity testing of chelonian aural abscesses. If the bacteria isolated are only a portion of the flora present and may not be contributing substantially – if at all – to the pathology of the lesion, choosing antibiotics on the basis of their sensitivity profile may be pointless. Further, if antibiotics are effective for aural abscesses (which is currently unknown), then choosing a narrow-spectrum antimicrobial on the basis of sensitivity testing for a bacterium that may be incidental to, or only a minor contributor to, the pathology may be harmful for the patient if a broader-spectrum anti-microbial would have been more beneficial.

These recommendations are based on evidence from free-living Eastern box turtles and captive emydid turtles, and it is not known to what extent they apply to other species, including captive testudinid tortoises, the most common chelonians seen in practice in the UK.

* The evidence does not indicate whether systemic antimicrobials are of benefit in uncomplicated cases, in addition to standard treatment of surgical debridement and topical antiseptic/antimicrobial treatment and addressing any husbandry deficiencies. Thus, we recommend that systemic antibiotics not be used for uncomplicated aural abscesses unless and until evidence should become available that they are of benefit. If the animal is systemically ill, that should be addressed, and that may require systemic antimicrobial therapy.
* For topical application, there is no evidence addressing whether antimicrobial use is of any benefit over antiseptic. Thus, we recommend use of topical antiseptic rather than antibiotic.
* If antibiotics are to be used, because the degree to which any one isolated bacterium contributes to the pathology of aural abscesses is unknown, the evidence does not indicate whether or not selecting antibiotics on the basis of culture and sensitivity testing would be of benefit over use of empirically selected antibiotics.
* If antibiotics are to be used, because no one species is consistently isolated from aural abscesses, and a broad range of bacteria have been associated with these abscesses, empirical choice would require a broad-spectrum antibiotic effective against Gram negative, Gram positive, aerobic and anaerobic bacteria. Therefore, for empirical, first-line use, the basic principles of responsible antimicrobial usage should be followed, i.e., first-tier antibiotics should be used, and higher-tier antibiotics (e.g., fluoroquinolones and third- and fourth generation cephalosporins) avoided. Trimethoprim-sulphonamide (first-tier) is a rational first-line choice, although would have limited effect against *Pseudomonas aeruginosa* ( Tang *et al* 2020).
* In emydid turtles, *P. testudinis* and *Pseudomonas* spp. are commonly isolated. Although it is not known if they play a significant role in the pathology of aural abscesses, precautionarily, if antibiotics are to be used, the spectrum of effectiveness of the antibiotic should include these bacteria

**ORAL INFECTIONS**

Stomatitis may occur for a variety of reasons in the captive reptile, but is often secondary to trauma or an underlying deficit in husbandry or diet leading to immunosuppression and overgrowth of commensal bacteria (Hedley, 2016). In snakes, the normal oropharynx contains a wide range of aerobic and anaerobic bacteria including both gram-positive and gram-negative isolates (Dipineto *et al.*, 2014). Gram-negative bacteria are usually the most common organisms to overgrow in cases of stomatitis including *Pseudomonas, Aeromonas, Proteus*, and *Escherichia coli* (Draper *et al.*, 1981). Anaerobic bacteria may also be isolated, such as *Bacteroides, Fusobacterium, Clostridium*, and *Peptostreptococcus* (Stewart, 1990). Atypical bacteria such as *Mycobacterium* *spp* (Quesenberry *et al.*, 1986), fungal and viral infections should also be considered.

Following initial assessment, oral lesions should be sampled for microbiological culture and sensitivity to help guide selection of appropriate antibiotic therapy. Ideally, the animal should be anaesthetised to determine the extent of infection and to facilitate full sampling, ideally from below the gingiva to avoid oral flora contamination (for bacterial and fungal culture and histopathology), debridement and cleaning of the lesions with a topical antimicrobial such as 0.05% chlorhexidine solution (Brown *et al.*, 2019). Distinguishing normal from pathogenic flora in cases of stomatitis is difficult, although if growth is “profuse” or if the overgrowth is of a single bacterium, then an overgrowth of pathogenic flora is more likely. It is likely that typical sampling and culture results under-represent the true prevalence of anaerobes relative to aerobes. Regular topical cleaning should be performed whilst awaiting results and topical silver sulfadiazine creams can be useful (De Voe, 2019). If systemic antibiosis is required, pending culture results in addition to topical treatment, empirical treatment may need to be initiated with a trimethoprim-sulphonamide.

In lizards with acrodont dentition, oral infection may also be seen associated with periodontal disease. Inappropriate dietary items such as fruit are thought to result in the development of plaque, subsequent bacterial colonization, and then gingivitis and calculus formation *(*Mott *et al.,* 2021). Initial bacteria involved are normally gram-positive aerobic cocci, but as the disease progresses, anaerobic bacteria, gram-negative bacteria and spirochetes may also be involved, eventually leading to osteomyelitis. Teeth should be assessed and scaled under anaesthesia and gingival pockets should be irrigated with dilute chlorhexidine flushing (McCracken, 1999). Systemic antibiotic treatment is only required if infection has affected underlying bone. These cases will need prolonged treatment for > 6 weeks, so antibiotic selection should always be based on culture and sensitivity for optimal results.

In chelonians, normal oral microflora is usually composed of Gram negative bacteria including *Pseudomonas, Klebsiella, Aeromonas and Salmonella spp* (Heynol *et al.*, 2015)*.* In chelonians, a strong primary viral association has been made and stomatitis is usually part of a stomatitis-rhinitis-conjunctivitis complex. In tortoises, herpesviruses and ranavirus appear particularly common and viral screening is always recommended (Soares *et al.*, 2004; Johnson *et al.*, 2008). Clinical signs with either virus includes ulcerative to diptheroid necrotising stomatitis, with rhinitis and conjunctivitis. Concurrent infection with organisms such as *Mycoplasma* may lead to immunosuppression and secondary stomatitis or recrudescence of herpesvirus infection. Culture of samples from oral lesions may guide treatment; common secondary bacterial agents include *Pseudomonas, Klebsiella, Aeromonas* and *Salmonella spp*. and opportunistic fungal agents are not uncommon (Musgrave and Mans, 2019). Treatment is multifactorial and does not solely rely on antibiotic therapy, but supportive care and addressing any underlying husbandry deficits and concurrent disease. Topical treatment should be considered as for snakes and lizards. Systemic antibacterial and/or antifungal treatment to treat secondary infections should be based on culture and sensitivity results and *Mycoplasma* PCR testing (see **Respiratory infections**).

**RESPIRATORY INFECTIONS**

*Chelonians*

Upper respiratory tract infections are commonly seen in chelonians, often secondary to husbandry or nutritional deficits. Predisposing factors such as low temperatures, vitamin deficiencies (in particular hypovitaminosis A) and inadequate ventilation should all be considered in addition to any other stressors or causes of immunosuppression such as concurrent disease.

The most common presentation in the terrestrial tortoise is “Runny nose syndrome” or stomatitis-rhinitis-conjunctivitis complex. Signs may include nasal discharge, conjunctivitis, stomatitis, lethargy and anorexia. Various infectious agents may be involved but the most common pathogens isolated are chelonian herpesviruses, ranaviruses and *Mycoplasma* spp(Soares *et al.*, 2004; Johnson *et al.*, 2008; Brown *et al.*, 2004). All these pathogens are widespread in the captive population and have also been identified in wild chelonians. Coinfection with ranaviruses, herpesviruses and *Mycoplasma* spp has been documented and a diagnosis of one disease, does not rule out the presence of other aetiologies (Sim *et al.,* 2016). Disease outbreaks often occur when species are mixed.

The clinical signs of ranavirus infection in chelonians are often similar from those of herpesvirus and *Mycoplasma* spp., highlighting the importance of diagnostic testing and achieving a definitive diagnosis (Benetka *et al.,* 2007; Sim *et al.,* 2016). Infection with herpesvirus leads to upper respiratory tract signs and in particular caseous plaques formation in the oral cavity, whereas *Mycoplasma* infection is more likely to be associated with conjunctivitis. However, both infections may occur concurrently. Diagnosis is usually based on PCR testing of a conjunctival or choanal swab, although serological testing is also possible. Treatment of the animal is often based on empirical antibiotic choice whilst awaiting results, supportive treatment including NSAIDs, mucolytics, nebulisation and correction of any predisposing factors. Disease is normally managed rather than cured. Fluoroquinolones, tetracyclines and macrolides (specifically clarithromycin and tulathromycin) have all been suggested to be useful to reduce clinical signs of *Mycoplasma* infection (Prezant, Isaza and Jacobson, 1994; Wimsatt *et al.*, 1999, 2008; Kinney *et al.*, 2014). Tetracyclines would be the preferred first-line antibiotic choice for systemic treatment, although nasal flushing with saline or topical antibiotics may also be helpful. Despite treatment, infected animals should be considered to be chronic carriers and clinical signs may flare up at times of stress and immunosuppression (Wimsatt *et al.*, 2008). Judicious use of antibiotics may be required on a case-by-case basis, preferably based in culture and sensitivity data as secondary sepsis or bacterial infections may develop in chelonians with viral aetiologies.

*Snakes and lizards*

In snakes, respiratory infections can often be seen in association with stomatitis. Owners may report wheezing, clicking sounds, bubbles from the mouth or nares or have seen the snake resting in abnormal postures, often stretched out. Alternatively, lethargy and anorexia may be the only clinical signs. Inadequate temperatures, poor hygiene and poor ventilation may all predispose to the overgrowth of commensal bacteria or fungi within the respiratory tract. Respiratory infections are less common in lizards but can occur for similar reasons. Normal respiratory flora in snakes include a range of gram positive and gram negative bacteria (Hilf, Wagner and Yu, 1990, Sonntag *et al.,*2020). *Salmonella, Mycoplasma, Pseudomonas, Klebsiella, Proteus, Aeromonas* have all been associated with clinical disease (Penner *et al.*, 1997; Cushing *et al.*, 2011; Schmidt *et al.*, 2013). However, atypical bacterial infections such as mycobacteriosis (Hernandez-Divers & Shearer, 2002), fungal or viral infections should also be considered, especially if a new snake has been added to the collection in recent months.

Antibiotic resistance has been recognised as a significant problem in snakes with respiratory disease, with less than 50% of bacteria isolated being sensitive to a variety of commonly used antibiotics (Sonntag *et al.* 2020). Sampling for culture and sensitivity is therefore strongly recommended. This may be via a tracheal wash or performed endoscopically (Schumacher, 2011). Treatment usually involves a prolonged course of systemic antimicrobial therapy (minimum 2 weeks – several months) based on culture and sensitivity results. Whilst awaiting results, empirical treatment may be started with doxycycline or trimethoprim-sulfamethoxazole.

**SKIN AND SHELL INFECTIONS**

A variety of infectious agents may be involved in skin infections in reptiles including both bacteria and fungi. Usually, these infections occur secondary to immunosuppression and are often linked to poor husbandry, although traumatic injuries can be another common cause especially following predator attack in chelonia.

*Snakes and lizards*

In snakes, primary bacterial skin infections are rarely reported, but overgrowth of commensal organisms is relatively common leading to bacterial vesicular dermatitis (“scale rot”). It is therefore important to always treat the underlying issues. A wide range of gram-positive, gram-negative, and anaerobic bacteria may be isolated in particular *Providencia rettgeri, Pseudomonas aeruginosa, Morganella morgannii, Enterococcus spp, Clostridium spp,* and *Salmonella spp* (Maas *et al.*, 2010)*.* Treatment should follow cultures from skin biopsies, as many environmental contaminants can be isolated from superficial samples and a prolonged treatment course is often necessary. While awaiting culture results, topical treatments such as silver sulfadiazine cream may be indicated.

In lizards, primary skin disease is also rare with the exception of *Devriesia agamarum*. This bacterium mainly appears to affect desert lizards but may be carried asymptomatically by bearded dragons. Lesions are seen mainly around the mouth but can be anywhere on the body and also have a yellow crusting appearance. Diagnosis is based on culture and histopathology of skin biopsies. Disease can be treated with ceftiofur at 5mg/kg SID for 12 days and resolved successfully if detected at an early stage (Hellebuyck *et al.*, 2009). *Devriesia agamarum* is resistant in the environment, with the potential to remain infective for over 5 months in humid sand, emphasising the importance of environmental control as well as antibiotics based on culture and sensitivity (Hellebuyck *et al*.,2011). It is also vital to differentiate between bacterial skin disease and fungal disease with dermatomycoses being caused by obligate pathogenic fungi of the families *Onygenaceae* (formerly referred to as *Chrysosporium* anamorph of *Nannizziopsis vriesii*)and *Clavicipitaceae* in captive and free-living reptiles (Schmidt, 2015).

*Chelonians*

Septicaemic cutaneous ulcerative dermatitis (SCUD) affects freshwater chelonians and is caused by a variety of isolates such as *Aeromonas hydrophila, Citrobacter freundii and Serratia* and *Mycobacterium spp.* (Hernandez-Divers *et al.*, 2009; Oros *et al.*,2003). These bacteria gain access through abrasions to the skin and shell, or as a consequence of a lack of access to a basking area resulting in continuous soaking of the skin and shell. Chronic stress and immunosuppression due to suboptimal husbandry conditions (such as poor water quality) play an important role as well. Systemic treatment should be based on bacterial and fungal culture results from sampling affected ulcers as there has been shown to be marked variation in antimicrobial susceptibility (Chen *et al.*,2015). Alternatively, if septicaemia is present/suspected, antibiotic selection should be based on blood culture (see **Sepsis** section). In a study investigating the prevalence of *Citrobacter* spp. from pet turtles and their environment, *Citrobacter* isolates were susceptible to sulfamethoxazole/trimethoprim, tetracycline as well as amikacin, ceftriaxone, ciprofloxacin and imipenem (Hossain *et al.,* 2017). Topical treatment can also be useful and debridement and packing lesions with a waterproof paste (e.g. Orobase®) mixed with an aminoglycoside has been recommended (Fraser and Girling, 2019), although with increasing concerns regarding the impact of antimicrobial use on the emergence of antimicrobial resistance the authors suggest using topical silver sulfadiazine pending sensitivity data

Tortoises are more likely to present with traumatic injuries, especially following predator attack. In one study, study 70.9% of tortoises bitten by dogs were infected with recognised potential pathogens (Pellett 2016). Fungi and yeasts were always found in combination with bacteria and never detected as the sole pathogen. Bite wounds were found to be polymicrobial, with both Gram-positive and Gram-negative isolates yielded, with potential pathogens cultured to include *Pseudomonas* spp., *Bacillus spp., E coli, Enterobacter cloacae, Aeromonas hydrophilia, Klebsiella oxytoca* and*, Clostridium* spp. Isolates cultured within bite wounds were isolates found in canine saliva but may also originate from the skin of the tortoise and from the environment. Some isolates cultured were opportunistic environmental bacteria with the potential to cause infection upon breaching of the skin. Based on culture from bite wounds, anaerobes appeared to be under-represented but were frequently isolated in canine saliva from other studies (Allaker *et al.,* 1997; Dewhirst *et al.,* 2012; Abrahamian and Goldstein, 2011; Goldstein, 1992). In the dog-bite study, the prevalence of anaerobes was low but does not rule out anaerobic infection due to the difficulty of growth, requiring anaerobic media, long incubation periods and further biochemical tests for identification of the organisms (Citron *et al.,* 1996). Based on the antimicrobial spectrum of activity, enrofloxacin may be an appropriate choice for tortoises presenting with dog bite wounds, especially if *Pseudomonas* spp. were isolated, however it should be reserved until culture and sensitivity data is obtained. Although anaerobes were not frequently isolated in this study, due to the frequency they were reported in the literature in the normal canine microbiota, it is recommended to provide cover with metronidazole. In the thesis, ‘Culture of shell dog bite wounds in terrestrial chelonia’, (2016) only ceftazidime and enrofloxacin were investigated. Culture results from bite wounds of chelonia were retrospectively reviewed and these yielded many isolates susceptible to doxycycline (*Sphingomonas paucimobilis, Staphylococcus epidermis, Aeromonas hydrophila, Pasteurella multocida, Leclercia adecarboxylata, Moraxella* spp., *E coli, Pantoea* spp., and *Serratia* spp. *E.coli* was the most common isolate from bite wounds reported in the thesis. If deemed necessary , such as if the incident occurred more than six hours before presentation (Mitchell 2002), if the injury is severe such as full thickness fractures of the carapace and plastron or if the wounds are already infected, empirical treatment may be started with doxycycline, after wound irrigation using sterile saline, pending culture and sensitivity results.

**SEPSIS**

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated

host response to infection, which may result in septic shock (Rhodes *et al.*, 2017). Guidelines have been established in human medicine for the best treatment of sepsis and septic shock and veterinary guidelines have subsequently been developed based on these (Keir and Dickinson, 2015). Intravenous antibiotics are recommended within the first hour of recognition of either septic shock or severe sepsis, whilst blood culture results are pending. It is also recommended that initial antimicrobial therapy should ‘‘include one or more drugs that have activity against the likely pathogens and penetrate into the tissues presumed to be the source of sepsis.’’

The challenge in a reptile is that the diagnosis of sepsis is often only suspected based on clinical signs such as petechiation and ecchymoses on the ventrum or an abnormal red colouration to the plastron and disease is unlikely to be detected at such an early stage (Fraser and Girling, 2019). Sepsis can also be suspected from haematology results including leukocytosis with a left shift, blood smear with bacterial colonies or phagocytosed bacteria in the white blood cells. Septic shock is also not well recognised. Blood cultures may be performed although care should be taken in interpreting results; especially if small volumes of blood are used. One study in western ratsnakes isolated bacteria such as *Morganella morganii, Citrobacter freuundii* and *Proteus* in blood cultures from apparently healthy snakes ( Waugh *et al.*, 2017). However, a heavy pure growth of a single bacterial species along with white blood cell changes may be suggestive of sepsis, although sometimes the reptile is too debilitated to mount an effective immune response. Disease is usually only confirmed on bacterial culture post-mortem.

Bacteria which have been isolated from cases of sepsis in snakes include *Salmonella enterica*, *Enterobacter cloacae*, *Aeromonas hydrophila*, *Pseudomonas* and *Proteus* (Esterabadi *et al.*, 1973; Soveri, 1984; Coutinho *et al.*, 2001; Abba *et al.*, 2016). In lizards, bacteria include *Streptococcal spp.* in Emerald monitors, *Listeria monocytogenes* in the bearded dragon and *Devriesea agamarum* in a variety of species (Hetzel *et al.*, 2003; Girling and Fraser, 2004; Martel *et al.*, 2008). In chelonians, there have been further bacteria isolated from cases of sepsis including *Helicobacter*, *Aeromonas hydrophila, A. veronii, Citrobacter freundii, Morganella morganii, Edwardsiella tarda,* *Wohlfahrtiimonas chitiniclastica, Chryseobacterium sp., Comamonas sp.* and even *Mycobacterium chelonae* (Murray *et al.*, 2009; Stacy and Wellehan, 2010; Chung *et al.*, 2017).

With this wide variety of potential bacterial pathogens, broad-spectrum antibiotic cover is recommended. For Gram negative sepsis an aminoglycoside would be the chosen antibiotic and for Gram positive sepsis ampicillin can be used. Ideally given by the intravenous route in cases where sepsis is suspected, in addition to supportive treatment.

**CONCLUSION**

Antibiotic resistance is an increasing problem and careful consideration should be given to the use of antibiotics in reptiles as for other species. Choice of antibiotics should always be carefully considered based on knowledge of the most likely pathogen(s) if culture and sensitivity results are not available. Based on the literature search performed, practice guidelines such as those suggested in the table below (**Table 3**) should ideally be formulated for each species to optimize therapy and minimize inappropriate use of antibiotic.

**TABLE 3 – EXAMPLES OF FIRST-LINE ANTIBIOTIC OPTIONS FOR COMMON CLINICAL SCENARIOS IN REPTILES**

|  |  |  |  |
| --- | --- | --- | --- |
| **Body system** | **Situation of use** | **Is systemic antibiotic treatment necessary?** | **Antimicrobial first-line choice pending sensitivity results** |
| **Oral** | Stomatitis in snakes | Yes | Trimethoprim-sulphonamides |
| **Respiratory** | Stomatitis-rhinitis-conjunctivitis complex in chelonians | Yes | Tetracyclines |
|  | Bacterial pneumonia in snakes | Yes | Doxycycline or trimethoprim-sulphonamides pending sensitivity results |
| **Skin infections** | Superficial lesions | No | Topical silver sulfadizine |
|  | Bite wounds or deeper lesions | Yes | Doxycycline |
| **Ear infections** | Aural abscesses in chelonians | If surgical removal incomplete or associated with systemic signs | Trimethoprim-sulphonamides |
| **Ocular infections** | Conjunctivitis / keratitis | No | Topical chloramphenicol |
|  | Subspectacular abscesses | Yes | Topical aminoglycoside (gentamicin/amikacin) |
| **Miscellaneous** | Sepsis | Yes | G+ ampicillin  G- aminoglycosides |

In view of the increased concerns about antimicrobial resistance, clinicians must ensure they practice good antimicrobial stewardship. The key points to consider before prescribing antimicrobials in reptile include:

* Confirm the existence of a bacterial infection. Empirical antibiotic usage should be discouraged. Bacterial cultures should be performed to identify the bacteria involved. If this is not possible, a cytology and a Gram stain should at least be performed before initiating any antimicrobial treatment. Exceptions to this would be the use of antibiotics to prevent infection in immunocompromised animals, animals with severe trauma or wounds and perioperative use.
* Antibiotic selection should be based on culture and sensitivity results or at least Gram stain identification. The narrowest spectrum of activity should be selected. First-line antibiotics should be used for initial treatment and second-line antibiotics should be reserved if culture and sensitivity indicate the need for their use.
* Body temperature and hydration status can affect drug absorption, metabolism and excretion. Antimicrobial treatment should ideally be delayed until the reptile patient is rehydrated and at the preferred range of body temperature for the species. The exception to this would be in the case of sepsis where treatment should be initiated immediately.

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