

Combination treatment of a pseudomonad abscess in a western black-tailed rattlesnake *Crotalus molossus molossus*: a case report

M. LUKAC, K. MATANOVIC, L. BARBIC, B. SEOL

Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

ABSTRACT: A three-year-old male western black-tailed rattlesnake (*Crotalus molossus molossus*), which was refusing food and losing weight was presented with a swelling on the right side of the head below the eye. An abscess was suspected and treatment with subcutaneous enrofloxacin was started immediately. After identification of the causative agents as *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, and following susceptibility testing, enrofloxacin treatment was replaced with marbofloxacin, intralesional gentamicin/betamethasone treatment and gentamicin administered subcutaneously at an increased terrarium temperature of 35 °C. Seven days later, the formed pus plug was debrided and a combined marbofloxacin/gentamicin/betamethasone treatment was continued for an additional seven days. The swelling disappeared. Marbofloxacin was continued for ten more days, after which time microbiology tests were negative for *S. maltophilia* and *P. aeruginosa*. The animal began to eat and gain body weight. To our knowledge, this is the first report of an abscess treatment in a rattlesnake and the first to demonstrate the effective treatment of a *S. maltophilia*-induced infection with a combination of marbofloxacin and gentamicin.

Keywords: abscess; marbofloxacin; gentamicin; *Pseudomonas aeruginosa*; *Stenotrophomonas maltophilia*

Data on the normal bacterial flora of snakes in captivity is very scarce. It is known that the normal flora is comprised primarily of gram-positive bacteria, predominantly corynebacteria and coagulase-negative staphylococci, and to a lesser extent gram-negative bacteria such as *Pseudomonas* species, including *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* (Blaylock 2001; Hejnar et al. 2007). These bacteria are thought to be present in the oral cavity of approximately one third of healthy snakes (Hejnar et al. 2002). As such, these bacteria are often considered opportunistic pathogens (Hilf et al. 1990). *S. maltophilia* is a multi-resistant, aerobic, ubiquitous gram-negative bacillus, which lives in humid areas (Senol 2004). *Pseudomonas* species are very common isolates from water vessels in vivariums (Ross and Marzec 1984). These bacteria only rarely cause clinically manifest disease in completely healthy

animals. In less competent animals (kept under suboptimal conditions, injured, etc.), these gram-negative bacteria may overgrow the population of gram-positive bacteria and cause disease (Draper et al. 1981; Harris and Rogers 2001). These pathogens are often resistant to most of the commonly used antimicrobials. Susceptibility has been tested to those antibiotics that are routinely used to treat gram-negative flora in humans, but are also very effective in animals (Ross and Marzec 1984; Frye 1991). When considering aminoglycosides and other antibiotics, it should be borne in mind that they are nephrotoxic and that their effectiveness at 30 °C is significantly lower than at higher temperatures. This particularly applies to *S. maltophilia* (Denton and Kerr 1998; Hejnar et al. 2002). When treating reptiles, it is advisable to increase the ambient temperature to close to the upper limit of the recommended range (Frye 1991).

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Case description

A 3-year-old male western black-tailed rattlesnake (*Crotalus molossus molossus*) weighing 560 grams was presented with a swelling on the right side of the head just below the eye (Figure 1). The snake had refused food for three weeks and was losing weight. Clinical examination did not reveal any other diseases.

The snake was kept by the same owner for the last 2 years, in a terrarium sized 1 × 0.6 × 0.6 m (length × width × height). Daytime and night temperatures in the terrarium were 25–30 °C and 20–22 °C, respectively, and relative humidity was 60–70%. The lighting regime was 12 hours of light and 12 h of darkness.

A general clinical examination of the patient under manual restraint revealed a mild gingival redness on the right side of the head, below the site of the injury. Palpation of the swelling indicated that this could be an abscess; therefore, treatment was started with enrofloxacin at 10 mg/kg of body weight q24hr *s.c.* (Baytril[®], Bayer HealthCare, Animal Healthcare Division, Shawnee Mission, Kansas, USA) and the terrarium temperature was increased to 35 °C. On the third day of the enrofloxacin treatment, the abscess matured, the lesion was opened and, after sampling for bacteriological analysis, was rinsed with 0.05% antiseptic chlorhexidine solution.

A swab of an abscess capsule was inoculated on Columbia agar (Biolife, Milan, Italy) and after

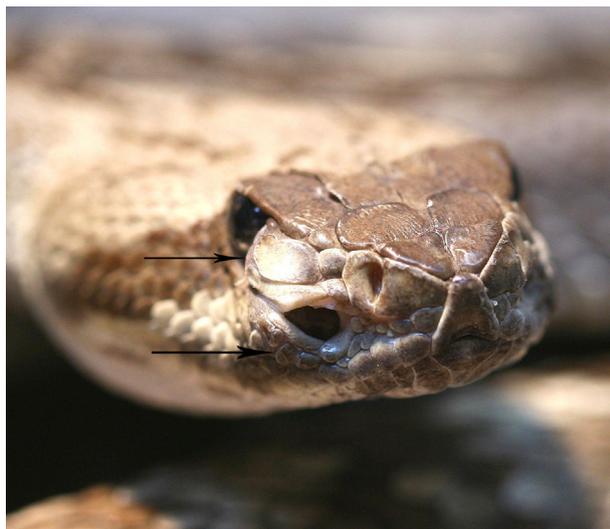


Figure 1. A swelling under the right eye seen macroscopically on the first examination. Arrows point to a prominent swelling affecting parts of the eye, nose and nasolacrimal channel

18 hours of incubation at 27 and 37 °C two types of colonies grew. Further identification revealed (a) catalase- and oxidase-positive, pigmented *Pseudomonas*-like colonies, and (b) catalase-positive, oxidase-negative colonies. Isolates were subsequently identified as *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* using commercial strips for biochemical identification of bacteria (API 20 NE System, bioMérieux S.A., Marcy-l'Etoile, France). The susceptibility of the isolated bacteria to antibiotics was determined using the Kirby-Bauer disk diffusion antibiotic susceptibility test on Mueller-Hinton agar (Becton, Dickinson & Co., Cockeysville, Maryland, USA) at 27 °C and 37 °C as per CLSI guidelines (CLSI 2010). The isolate of *S. maltophilia* was susceptible to enrofloxacin, ciprofloxacin and marbofloxacin and resistant to gentamicin, amikacin and ceftazidime at 27 °C. At 37 °C this bacterium was susceptible to marbofloxacin, enrofloxacin, ciprofloxacin, gentamicin, and amikacin, and resistant to ceftazidime. *P. aeruginosa* was susceptible to marbofloxacin, enrofloxacin and ciprofloxacin and resistant to amikacin and ceftazidime at 27 °C. At 37 °C this bacterium was sensitive to marbofloxacin, enrofloxacin, ciprofloxacin, gentamicin and amikacin, and resistant to ceftazidime.

On the ninth day after the start of antibiotic administration, the lesion was again thoroughly rinsed with 0.05% chlorhexidine. A combination of gentamicin and betamethasone (Garasone[®] drops, Schering-Plough Labo N.V., Brussels, Belgium) was then administered locally while enrofloxacin was replaced by marbofloxacin (Marbocyl 2%, Vetoquinol SA, Lure cedex, France) at 10 mg/kg *s.c.* daily. Treatment with marbofloxacin *s.c.* and Garasone[®] drops locally was continued for the next seven days. In addition, gentamicin (Gentocin[®], Schering-Plough Animal Health, Kenilworth, New Jersey, USA) was administered on three occasions during this period at a dose of 4 mg/kg *s.c.* on the first occasion, i.e. on the same day when marbofloxacin was introduced, followed by two doses of 2 mg/kg, each three days apart. On day seven of marbofloxacin/Garasone treatment, the formed pus plug was removed from the nasolacrimal channel and the channel was rinsed with ethacridine lactate (Rivanol 0.1%, Zagreb Pharmacy, Zagreb, Croatia). The treatment with marbofloxacin *s.c.* and Garasone locally was continued for the next seven days. The swelling disappeared (Figure 2) and the animal received five additional marbofloxacin



Figure 2. Completely resolved condition

doses *s.c.* every 48 hours. A new swab was taken for culture and the tests were negative for both *S. maltophilia* and *P. aeruginosa*. The animal began to eat and an increase in body weight was observed. The overall treatment period was approximately one month.

DISCUSSION AND CONCLUSIONS

Pseudomonad-type bacteria are not uncommon in the oral cavity of snakes. However, under unfavourable conditions, if the animal is immunocompromised or, in the case of *P. aeruginosa* if water vessels are not regularly cleaned, these bacteria may cause clinically manifest disease. *S. maltophilia* is likely a part of the normal bacterial flora of the oral cavity and cloaca in approximately 10% of wild snakes and in healthy snakes kept in captivity (Draper et al. 1981; Ross and Marzec 1984; Hejnar et al. 2007). Pseudomonad-type bacteria including *S. maltophilia* are considered to be the most common causes of infections in reptiles. Clinical symptoms include ulcerative stomatitis, abscesses, septicemic ulcerative skin changes, respiratory infections and necrotising dermatitis (Draper et al. 1981; Ross and Marzec 1984; Frye 1991; Harris and Rogers 2001). Infections caused by pseudomonad-type bacteria (*P. aeruginosa* and *S. maltophilia*) are more difficult to treat because they exhibit considerable resistance to many currently used antibiotics (Seol et al. 2002; Hejnar et al. 2007).

Based on the results of susceptibility testing, we decided to use marbofloxacin as its effectiveness against both bacterial isolates was demonstrated at 27 °C and 37 °C *in vitro*. In addition, marbofloxacin is less irritating than enrofloxacin in long-

term treatment, which was anticipated in this case, considering the persistence of pseudomonad-type infections. To increase the efficacy of the treatment, gentamicin was administered concomitantly on three occasions, three days apart, with a higher dose on the first occasion and lower doses on the second and third occasions. This was carried out to increase the effect of marbofloxacin, because both bacterial isolates were also shown to be sensitive to gentamicin at 37 °C. In addition, gentamicin and betamethasone were administered locally so as to exert direct antibiotic and anti-inflammatory effects at the site of infection. During the treatment, the animal was kept at an ambient temperature close to the upper limit of the recommended range (35 °C) to increase the effectiveness of these antibiotics (Frye 1991). In conclusion, *S. maltophilia*, present in the oral cavity of one third of healthy snakes, may cause clinically manifest disease if snakes are kept under suboptimal conditions such as low temperature, excessive humidity and dirty water vessel in the terrarium (Needham 1986) like in the case described here. This pathogen demonstrates high persistence and resistance to many commonly used antimicrobials, particularly at lower terrarium temperatures. We demonstrated that such an infection can be effectively treated with marbofloxacin and gentamicin and that these antibiotics are effective at the upper limit of the optimal ambient temperature for this snake species. To our knowledge, this is the first report of an abscess treatment in a rattlesnake and the first to demonstrate the effective treatment of *S. maltophilia*-induced infection by a combination of marbofloxacin and gentamicin.

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Corresponding Author:

Maja Lukac, DVM, University of Zagreb, Faculty of Veterinary Medicine, Department of Avian Diseases with Clinic, Heinzelova 55, Zagreb 10000, Croatia
Tel. +3851 2390 410, Fax +3851 2390 280, E-mail: maja.lukac@vef.hr
