Case report

FATAL DISSEMINATED PSEUDOMONAS AERUGINOSA INFECTION IN A CAPTIVE GREEN IGUANA *(IGUANA IGUANA)*

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Infections with various bacteria, especially gram-negative aerobes, are a well-recognized problem in captive cold-blooded animals with immunocompromised health status, or in those kept under poor conditions. *Pseudomonas* is one of the most represented genera. Here, we present a case of fatal disseminated infection caused by *Pseudomonas aeruginosa* in a captive green iguana kept at the "Pionirska dolina" Zoo in Sarajevo, Bosnia and Herzegovina. At necropsy, severe stomatitis, pneumonia, hepatitis and nephritis, accompanied with focally extensive dermatitis were observed. Histopathology revealed multifocal necrosis in various visceral organs. Culture and subsequent MALDI-TOF MS analysis were conducted to identify the isolate as *P. aeruginosa*. Antimicrobial susceptibility testing revealed a wide susceptibility of the isolate, however applied therapy was instilled too late in the presented case. This case demonstrates the significance of timely and accurate identification, and antimicrobial susceptibility testing of bacterial isolates implicated in the pathology of captive reptiles. The importance of monitoring the adequate environmental conditions (enclosure temperature, humidity and conformation), health status and possible clinical signs of illness are highlighted.

Key words: Green iguana, Iguana, Pseudomonas aeruginosa, MALDI-TOF MS, histopathology

INTRODUCTION

Pseudomonas is a ubiquitous genus which consists of Gram-negative aerobic bacteria that are commonly detected in the environment and as a constituent of normal microbiota in various animal species [1]. Opportunistic and concomitant infections are often caused by *Pseudomonas aeruginosa* in cold-blooded animal hosts with a compromised

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immune system. Malnourished animals or those held in poor conditions, such as suboptimal environmental temperatures and humidity are prone to *Pseudomonas* sp. infection [2-4]. *P. aeruginosa* is known for its zoonotic potential where humans are usually infected through inhalation or potential contact with microorganisms from animal feces. Another possibility for infection are bite-wounds and scratches by the animals with *Pseudomonas* as a part of their normal oral microbiota [1,5].

Isolation of *Pseudomonas* species from reptiles have most commonly originated from diseased snakes [4,6], however, numerous isolates from lizards have been documented as well [1,7,8]. Lesions associated with *Pseudomonas* infection in reptiles are most commonly observed on the skin, oral cavity, tongue, lung and as systemic infections [4]. However, reports on lesions caused by this bacterium in green iguanas (*Iguana Iguana*) and other lizards are scarce [4,9-11].

Due to its zoonotic potential, multi-drug resistance, and possible involvement in the differential diagnosis of various lesions in lizards considerable attention should be directed toward this pathogen. Here, we describe a case of a fatal *P. aeruginosa* infection in a green iguana and provide data on the antimicrobial sensitivity of the isolate.

CASE PRESENTATION

In April 2015, a 7 years old, 180 cm long, male green Iguana (Iguana Iguana) from the Zoo "Pionirska dolina" in Sarajevo was submitted for necropsy at the Department of Pathology, Faculty of Veterinary Medicine, University of Sarajevo. Prior to presentation, the animal was transferred to a new terrarium previously inhabited by a boa constrictor. The iguana showed no clinical signs of illness and was fed with fruits and vegetables along with the weekly dose of vitamin and mineral supplements. Daytime temperature in the new terrarium ranged from 26 to 29 °C, and slightly lower i.e. 22 to 24 °C at night. Also, the terrarium was equipped with a heating pod and a bulb providing 32 to 35 °C. The humidity in the terrarium was 75 to 80%. Five days after the transfer, zookeepers observed decreased movements and reduced appetite of the animal. Two days later the condition of the animal did not improve and the iguana underwent systemic antimicrobial therapy with enrofloxacin (Enroxil 5%, Krka, Slovenia; 50 mg/mL, injection) in a dosage of 5 mg/kg per day. During the next five days, despite the continuous therapy the condition of the animal further deteriorated. Complete loss of the appetite, dryness of the skin, and multifocal ulcerations and crusts on lips and oral cavity were noted. The animal died on the eighth day of the onset of clinical signs of illness.

At necropsy, multifocal ulcerations with gray granular and proliferative tissue were observed on the oral cavity mucosa, gingiva and lips. Bilateral nasal serohemorrhagic discharge, and conjunctivitis were also visible (Figure 1a). In the middle of the tail at the right lateral aspect there was a 8 cm long and 1-2 cm wide grey to brown, hyperkeratotic, scabbed lesion with protruding patches of marginal skin. At cross section, the affected epidermis was slightly distended, grey and glistening as well as underlying muscle tissue (Figure 1b). Scant amount of clear liquid was found in the coelomic cavity and in the pericardium. Lung parenchyma was edematous. The liver was enlarged, tan to brown and firm. Multifocal white to gray up to 3 mm necrotic foci, often surrounded with a red rim were scattered throughout the parenchyma of the lung, liver and kidneys.

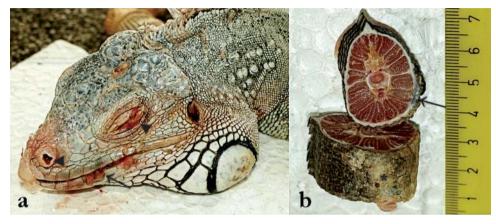


Figure 1. a. The head of a male green iguana infected with *Pseudomonas aeruginosa*. Note the protruding granular tissue at the edge of the mouth (arrow) and serohemorrhagic exudate from the nares and the eye (Arrow heads). b. Segment of the tail and cross section of a hyperkeratotic and exfoliating lesion (asterisk). At cross section grey white and glistening necrosis and degeneration of the dermis was evident (arrow).

Samples of parenchymatous organs and skin were collected for histopathology. Briefly, the samples were fixed in 10% neutral buffered formalin for 24–48 hours (h) and routinely processed for histopathology. Sections (4–6 μ m thick) were stained with hematoxylin and eosin (H&E) and examined under a light microscope. Skin sections were also stained with periodic acid-Shiff (PAS) stain in search for fungal elements.

Histopathology of the skin revealed focally extensive severe proliferation of the stratum corneum (orthokeratotic hyperkeratosis). In the underlaying thickened dermis, rarely there were multifocal perivascular moderate infiltrates of degenerated inflammatory cells, predominantly heterophils. Tail muscle fibers were degenerated and atrophic surrounded with moderate clear spaces (edema) which was also noticed in between the dermis and muscle (Figure 2). PAS stained sections revealed no fungal elements. Focally extensive ulcerative foci filled with eosinophilic exudate, granulation tissue and inflammatory cells, and covered with thick layered fibrinonecrotic material were visible on the commissure of the lips. Multifocally there were numerous basophilic colonies of rod-shaped bacteria.



Figure 2. Microphotograph of the skin lesion characterized with marked epidermal hyperkeratosis (arrows) and slightly expanded dermis with thickened degenerated collagenous fibers. Underlying muscle tissue was edematous with atrophic fibers. H&E.

In the parenchyma of the lung, liver and kidneys multifocal to coalescing necrotic areas were observed. They consisted of irregular necrotic areas filled with granular eosinophilic material, moderate number of degenerate inflammatory cells and numerous basophilic bacteria. Within necrotic foci lung septae were distended with infiltrates of numerous heterophils, macrophages and lymphocytes and occasional mild proliferation of connective tissue (fibrosis) (Figure 3a, b and c). In the rest of lung parenchyma, alveoli were filled with light homogeneous eosinophilic material (edema) and multifocal hemorrhages. Multifocally, glomeruli were expanded with proliferated hypereosinophilic mesenchyme with highly dilated arterioles, and infiltrated with a mild to moderate number of degenerated heterophils. Bowman spaces were distended and occasionally filled with homogenous eosinophilic material. Multifocal periglomerular proliferation of connective tissue which extends in to and expands the surrounding interstitium was observed. Throughout the kidney parenchyma necrotic tubulocytes were laden with granular orange to light brown material (hemoglobin), and tubular lumens were occasionally occluded with granular tissue debris. Mild multifocal proliferation of connective tissue admixed with irregular dark brown to black pigmented areas (melanin) were observed in the subcapsular spaces. There were small foci of mineralization multifocally in the interstitium. Hepatocytes were diffusely atrophic due to dilation of heavily hyperemic sinusoids. Multifocal moderate clear vacuoles were observed in the hepatocyte cytoplasm. Diffusely throughout the liver parenchyma aggregates of macrophages laden with brown to black granular material (hemosiderin) were evident.

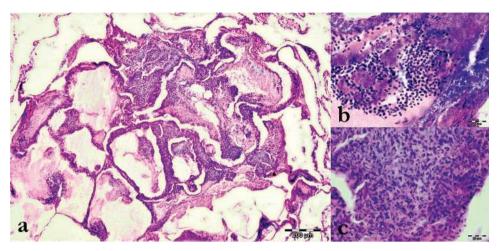


Figure 3. a. Section of the lung with expanding necrosis of the parenchyma composed of necrotic and distended septa and airway spaces filled with karyorrhectic debris and inflammatory cells. H&E. b. Focus of airways filled with inflammatory cells admixed with myriad coccobacillary bacteria. H&E. c. Necrotic foci are filled with degenerated inflammatory cells and karyorrhectic debris. H&E.

The samples of skin and lungs were submitted for bacteriological examination. Prior to inoculation onto blood agar (Condalab, Madrid, Spain) the samples were incubated in nonselective liquid medium Buffered peptone water (Condalab, Madrid, Spain) for 18±2 h at 37 °C. Later, 0.1 ml of the samples were inoculated onto blood agar and incubated at 37 °C for 24 h. The obtained cultured bacterial colonies (with green pigment and aromatic smell) were preserved and stored at -20 °C for further characterization. Later on the culture was submitted to the Institute of Microbiology and Parasitology, Veterinary Faculty (University of Ljubljana, Slovenia) for confirmation using the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (Microflex LT system; Bruker Daltonic, Germany). The isolate was identified as P. aeruginosa with a high confidence score value (2.377) which corresponds as highly probable bacterial species identification. The antimicrobial susceptibility of the isolated strain was determined by the disc-diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines (ref: Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals). The following antimicrobials were tested: amikacin (30 µg; Oxoid, UK), ceftriaxone (30 µg; BBL Becton Dickinson, France), ciprofloxacin (5 µg; BBL Becton Dickinson, France), enrofloxacin (5 µg; Mast Group, UK), gentamicin (10 µg; BBL Becton Dickinson, France), imipenem (10 µg; BBL Becton Dickinson, France), marbofloxacin (5 µg; Mast Group, UK), meropenem (10 µg; Oxoid, UK), piperacillin (100 µg; Oxoid, UK), polymyxin B (300 µg; Oxoid, UK), ticarcillin (75 µg; Oxoid, UK) and tobramycin (10 µg; Oxoid, UK). The isolate displayed susceptibility to amikacin, ciprofloxacin, enrofloxacin, gentamicin, imipenem, marbofloxacin, piperacillin, polymyxin B and tobramycin, reduced susceptibility for ceftriaxone and meropenem, and resistance to ticarcillin.

The sample of the skin was inoculated on Sabouraud dextrose agar (Condalab, Madrid, Spain), supplemented with chloramphenicol (100 mg/l) and incubated for five days at room temperature for fungal growth. No fungal growth was observed.

DISCUSSION

Many bacterial species are commonly isolated from reptiles, however, diseases often result from infections caused by gram-negative aerobic bacteria [4,8]. Among these bacteria, *Pseudomonas* is one of the most commonly isolated genera. In a recent study on bacteriology of skin lesions in 219 reptiles, 48 out of 306 isolates (15.69%) were *Pseudomonas* spp. [8].

P. aeruginosa has been frequently associated with cutaneous, oral, lingual, intestinal lesions, and pneumonia or even septicemia. Oral and chronic skin lesions may be the source of further spread of infection to the lungs resulting in pneumonia, or become systemic [4]. In this report all the above lesions associated with P. aeruginosa infection were present suggesting systemic infection with a fatal outcome. However, it is difficult to determine which of these was the primary one. The opportunistic nature of *P. aeruginosa*, its presence in the environment and common isolation from healthy iguanas [1,3,7], points to the existence of an underlying problem that compromised the immune status of this animal. According to zookeepers, few days prior to the onset of illness, the animal was transferred from its terrarium to another one previously used by the boa constrictor (Boa constrictor). The transfer itself was accompanied with changes in temperature, humidity and conformation of a new environment, might have triggered the stress and compromised the immune status that exposed the animal to infection. Also, the new enclosure was probably contaminated with P. aeruginosa from the boa constrictor because it is among bacterial species regularly isolated from both diseased and healthy boid snakes [12-14]. Therefore, if not cleaned and disinfected properly, the residual bacterial contamination of the terrarium might have been the source of infection for the newly introduced iguana. Furthermore, the tail lesions observed in this case, could have been the result of excessive heat, and represented a primary entry portal for P. aeruginosa infection. Thermal skin injuries in reptiles have been well described [15-17], however, researchers are still puzzled how and why these animals keep exposed to heat source despite high temperatures. Skin lesions in iguanas associated with different fungal infections have been commonly observed [18,19]. In the here presented case, we ruled out fungal etiology by both histopathology and negative culture results on specific medium.

Other lesions observed on internal organs (pneumonia, hepatitis and nephritis) are probably secondary in nature as was suggested elsewhere [4]. The inflammatory exudate in reptiles is very thick and gives rise to its difficult removal from tissues. It is in particular challenging in the respiratory tract having in mind the inability of reptiles to cough. Also, respiratory infection in reptiles goes with mild and subtle clinical signs, and most of the animals affected with respiratory diseases are presented with progressing lesions [20]. Hence, we conclude that the lung lesions described here were probably disseminated from the oral and upper respiratory tract through aspiration of the thick mucous exudate. Later on, progressing untreated lung lesions enabled the further dissemination of infection to the liver and kidneys. Even though *P. aeruginosa* was isolated from the skin and lung lesions, no blood was sampled and analyzed to confirm septicemia. Moreover, petechiae commonly present in septicemic *P. aeruginosa* infections in reptiles [11] were not observed in the presented carcass. In addition, viral etiology as a possible predisposing factor to bacterial pneumonia [4] could not be excluded in this case.

P. aeruginosa is intrinsically resistant to several antimicrobials: ampicillin, amoxicillinclavulanic acid, ceftriaxone, cefotaxime, ertapenem, chloramphenicol, kanamycin, neomycin, trimethoprim, tetracyclines and tigecycline [21]. The isolate from the present clinical case displayed susceptibility to various antimicrobials; amikacin, ciprofloxacin, enrofloxacin, gentamicin, imipenem, marbofloxacin, piperacillin, polymyxin B and tobramycin similar to *P. aeruginosa* isolates in other studies [1,7,8,22]. Regrettably, administration of enrofloxacin in this case failed to resolve the infection despite *in vitro* susceptibility of the detected isolate. These findings suggest that the therapy in the present case was too late, and the infection was already widespread to be successfully treated. Furthermore, antimicrobial susceptibility studies on aerobic bacteria isolated from reptiles indicated amikacin, ceftazidime, gentamicin or tobramycin as the treatment options to be considered in suspect cases of *Pseudomonas* spp. infection [22].

Moreover, this case shows that timely culture and specific determination of implicated bacterial species, as well as antimicrobial susceptibility testing of isolates are crucial for proper treatment of bacterial infections in reptiles [4,8]. Furthermore, in the era of widespread antimicrobial resistance of various bacteria, antibiotic sensitivity is mandatory in order to prevent further increase in resistance to already ineffective antimicrobials [8]. Selection of an appropriate antimicrobial is also important so that the resident gastrointestinal microbiota of reptiles, which participate in optimal digestion and immune functions, are maintained or minimally disrupted [23].

In conclusion, the lethal outcome in the presented animal resulted from severe disseminated lesions associated with *P. aeruginosa* infection. This case highlights the importance of controlling multiple environmental factors (enclosure temperature, humidity and conformation) along with proper sanitary actions which alone or in combination could predispose reptiles to various opportunistic bacterial infections. Constant monitoring of the health status, development of clinical signs, and prompt diagnostics (culture and antimicrobial sensitivity testing) of suspect bacterial cases should be established in order to avoid delayed and inadequate therapy as well the development of antimicrobial resistance.

Authors' contributions

JŠ performed the necropsy and histopathology and drafted the manuscript. ER carried out microbiology examination of samples, participated in the drafting and critically reviewed the manuscript. AK participated in drafting the manuscript. SH participated in microbiology examination of samples and participated in drafting the manuscript. MG performed MALDI-TOF MS analysis and antimicrobial susceptibility of isolates and critically reviewed the manuscript. VŠ performed clinical examinations. AA performed the necropsy and histopathology and critically reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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FATALNA DISEMINOVANA *PSEUDOMONAS AERUGINOSA* INFEKCIJA KOD ZELENE IGUANE (*IGUANA IGUANA*) U ZATOČENIŠVU

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Infekcije različitim bakterijama, posebno gram negativnim aerobima, su vrlo čest problem kod zatočenih hladnokrvnih životinja sa narušenim imunskim statusom ili onih držanih u lošim uslovima okoline. *Pseudomonas* je jedan od najzastupljenijih rodova. U radu je prezentovan slučaj fatalne diseminovane infekcije uzrokovane sa *Pseudomonas aeruginosa* kod zelene iguane iz zoološkog vrt "Pionirska dolina" u Sarajevu, Bosna i Hercegovina. Na obdukciji, ustanovljeni su teški stomatitis, pneumonija, hepatitis i nefritis kao i fokalno ekstenzivni dermatitis. Histopatološki je uočena multifokalna nekroza u različitim visceralnim organima. Kultivacijom i naknadnom MALDI-TOF MS analizom izolat je identifikovan kao *P. aeruginosa.* Testom antimikrobne osetljivosti ustanovljena je široka osetljivost izolata, međutim u prezentovanom slučaju terapija je aplikovana prekasno. Ovaj slučaj prikazuje važnost blagovremene i ispravne identifikacije, kao i testiranje antimikrobne osetljivosti bakterijskih izolata impliciranih u patologiju reptila držanih u zatočeništvu. Istaknuta je važnost praćenja adekvatnih uslova okoline (temperature staništa, vlažnost i konformacija), zdravstvenog statusa kao i mogućih kliničkih znakova bolesti.