

Vesicular dermatitis caused by multirresistant *Pseudomonas* spp. in the carpet python (*Morelia spilota* Lacepede, 1804) (Squamata: Pythonidae) – case report

Dermatite vesicular causada por *Pseudomonas* spp. multirresistente em píton carpele (*Morelia spilota* Lacepede, 1804) (Squamata: Pythonidae) – relato de caso

Dermatitis vesicular causada por *Pseudomonas* spp. multiresistente en pitón de alfombra (*Morelia spilota* Lacepede, 1804) (Squamata: Pythonidae) – reporte de caso

Received: 09/23/2021 | Reviewed: 10/02/2021 | Accept: 10/07/2021 | Published: 10/10/2021

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Abstract

Among the diseases that can affect snakes in captivity, infectious diseases are one of the main factors responsible for high morbidity and mortality rates. One of the main agents is *Pseudomonas* spp. that, besides being zoonotic, is generally multiresistant to several antimicrobials, representing a public health risk. This paper aims to report the occurrence of multidrug-resistant *Pseudomonas* sp. in *M. spilota* presenting vesicular dermatitis, as well as the microbiological, pathological and therapeutic description. In the care of a 7-year-old male carpet python, we noticed several vesicles containing a translucent viscous liquid, located in the skin of the entire body of the animal, in addition to some depigmented and elevated scales. Thus, material was collected from a freshly opened vesicle for bacterial culture and antibiogram. Blood was also collected for hematological and serum biochemical tests. Due to the suspicion of bacterial infection, treatment was instituted using enrofloxacin, ketoprofen, and potassium permanganate baths. After treatment, it was noticed that the vesicles had ceased, but after about ten days the animal died, suggesting systemic involvement. Hematological exams showed heterophilic leukocytosis, lymphocytosis, and monocytosis, besides an increase in AST. The results of the culture and antibiogram were available after the death of the animal, suggesting *Pseudomonas* spp. with resistance to several drugs. This paper describes the first report of vesicular dermatitis caused by multidrug-resistant *Pseudomonas* spp. in *M. spilota*, thus assisting in the medicine and ex situ conservation of these animals.

Keywords: Bacteria; Infection; Reptiles; Snakes; Zoonosis.

Resumo

Dentre as doenças que podem acometer serpentes em cativeiro, as doenças infecciosas são um dos principais fatores responsáveis pelas altas taxas de morbidade e mortalidade. Um dos principais agentes é *Pseudomonas* spp. que além de zoonótico, geralmente se apresenta multirresistente a vários antimicrobianos, representando um risco de saúde pública. O objetivo do presente trabalho é relatar a ocorrência de *Pseudomonas* sp. multirresistente em *M. spilota* apresentando dermatite vesicular, além da descrição microbiológica, patológica e terapêutica. No atendimento de uma piton carpete, macho de 7 anos de idade, percebeu-se diversas vesículas contendo um líquido viscoso translúcido, localizadas na pele de todo o corpo do animal, além de algumas escamas despigmentadas e elevadas. Dessa forma, foi coletado material de uma vesícula recém-rompida para a realização de cultura bacteriana e antibiograma. Também foi feita a colheita de sangue para exames hematológicos e bioquímica sérica. Devido a suspeita de infecção bacteriana, foi instituído o tratamento utilizando-se enrofloxacina, cetoprofeno e banhos com permanganato de potássio. Após o tratamento, percebeu-se que as vesículas haviam cessado, porém após cerca de dez dias o animal veio à óbito sugerindo acometimento sistêmico. Nos exames hematológicos verificou-se leucocitose por heterofilia, linfocitose e monocitose, além de aumento na AST. Os resultados da cultura e antibiograma foram disponibilizados após o óbito do animal, sugerindo *Pseudomonas* spp. com resistência para diversos fármacos. Este trabalho descreve o primeiro relato de dermatite vesicular causada por *Pseudomonas* spp. multirresistente em *M. spilota*, auxiliando assim para medicina e conservação ex situ desses animais.

Palavras-chave: Bactérias; Infecção; Répteis; Serpentes; Zoonose.

Resumen

Entre las enfermedades que pueden afectar las serpientes en cautiverio, las enfermedades infecciosas son uno de los principales factores responsables de las altas tasas de morbilidad y mortalidad. Uno de los principales agentes es *Pseudomonas* spp. que además de zonótico, generalmente se presenta como multirresistente a varios antimicrobianos, lo que representa un riesgo para la salud pública. El objetivo del presente trabajo es reportar la ocurrencia de *Pseudomonas* sp. multirresistente en *M. spilota* con dermatitis vesicular, además de descripción microbiológica, patológica y terapéutica. En una cita médica de una pitón de alfombra macho de 7 años, se notaron varias vesículas conteniendo un líquido viscoso translúcido, ubicadas en la piel de todo el cuerpo del animal, además de algunas escamas despigmentadas y elevadas. Por tanto, se recogió material de una vesícula recién rota para cultivo bacteriano y antibiograma. También se extrajo sangre para pruebas hematológicas y bioquímica sérica. Ante la sospecha de infección bacteriana se instauró tratamiento con enrofloxacino, ketoprofeno y baños con permanganato de potasio. Después del tratamiento, se notó que las vesículas habían cesado, pero después de diez días el animal murió, lo que sugiere una afectación sistémica. En los exámenes hematológicos se verificó leucocitosis por heterofilia, linfocitosis y monocitosis, además de aumento de AST. Los resultados del cultivo y del antibiograma estuvieron disponibles después de la muerte del animal, lo que sugiere *Pseudomonas* spp. con resistencia a varios fármacos. Este artículo describe el primer informe de dermatitis vesicular causada por *Pseudomonas* spp. multirresistente en *M. spilota*, contribuyendo así a la medicina y la conservación ex situ de estos animales.

Palabras clave: Bacterias; Infección; Reptiles; Serpientes; Zoonosis.

1. Introduction

The carpet python (*Morelia spilota* Lacepede, 1804) is a non-venomous snake belonging to the order Squamata and family Pythonidae. This species is native to Australia, Indonesia, and New Guinea. Reptile breeding in captivity has expanded considerably in recent years and nowadays many snake species are bred as pets, making the presence of these animals common in veterinary clinics. In addition, snakes are common in zoos and wildlife farms. (Stahl, 2002; Mitchell, 2004; Tallowin et al., 2017; Sala et al., 2019).

In these places, management errors such as inadequate temperature, humidity and ventilation are factors that are often associated with stress, reproductive seasons, and concomitant diseases, which favor the growth of opportunistic microorganisms. Therefore, it is known that infectious diseases have been indicated as one of the main factors responsible for high morbidity and mortality rates in captive snakes (Ferreira et al., 2012, 2014). Thus, snakes can be affected by several bacterial and fungal diseases, which in several cases are zoonotic, as in the case of *Aeromonas hydrophila*, *Salmonella* spp. and *Pseudomonas* spp. (Ebani & Fratini, 2005; Ferreira et al., 2012, 2014; Romero et al., 2015; Sala et al., 2019; Martins et al., 2017, 2021).

One of the most common bacteria found in these animals are species of the genus *Pseudomonas*, gram-negative and considered an opportunistic pathogen for both animals and humans. This can be detected in the oral and cloacal microbiota of

healthy and sick ofids, with higher frequency in those that live in captivity, representing a risk for the guardians and technicians who manage these animals. Possessing high pathogenicity, this bacillus is responsible for causing dermatitis, fibrosis, stomatitis, pneumonia, necrotizing enteritis, cloacitis, ear infections, and septicemia in reptiles, associated with high morbidity and mortality in these animals. However, the greatest difficulty in treating this infection is the high resistance to various classes of antibiotics, which represents a growing threat to public health worldwide (Rubin et al., 2008; Colinon et al., 2010, Breidenstein et al., 2011, Ferreira et al., 2012, 2014; Foti et al., 2013, Liu et al., 2017, Sala et al., 2019; Martins et al., 2021).

Thus, this paper aims to describe the first report of multidrug-resistant *Pseudomonas* sp. in *M. spilota* causing clinical manifestation characterized by vesicular dermatitis, as well as the microbiological, pathological, and therapeutic description.

2. Methodology and Case Description

A 7-year-old male carpet python (*Morelia spilota* Lacepede, 1804) had been transferred three months ago to the serpentarium of Ecopark Sol e Mar in Maragogi, Alagoas, Brazil. This animal was kept in plastic boxes and fed with young mercol rats (*Rattus norvegicus*) every 15 days from the vivarium. During treatment of this animal, multiple vesicles were noticed on the skin all over the body, in addition to some depigmented and elevated scales and apathy. These blisters burst as the animal moved and expelled a translucent viscous liquid (Figure 1).

Figure 1 - Lesions caused by *Pseudomonas* sp. in carpet python (*Morelia spilota*). A - Vesicles on dorsal scales, dorsal view; B - Lateral scales lifting, dorsal view; C - Ruptured vesicles, lateral view.



Source: Personal archive.

Thus, the material was collected by using a Swab of the content of a recently ruptured vesicle, was stored in Stuart's medium for further processing, and then sent to a clinical laboratory for bacterial culture and antibiogram. Blood was drawn from the spinal venous sinus for hematological and serum biochemical tests for uric acid, aspartate aminotransferase (AST), and alkaline phosphatase.

Due to the suspicion of bacterial infection, treatment was instituted using enrofloxacin 6mg/kg intramuscularly (IM) every 24 hours (SID), ketoprofen 2mg/kg IM every 48 hours (QOD), and potassium permanganate baths at a ratio of 1 tablet (3g) to 4 liters of water for 20 minutes SID. The entire treatment was carried out for 10 days (Carpenter, 2018). After three days of treatment, it was noticed that the vesicles had ceased, but after about ten days the animal died.

The hematological exams showed leukocytosis ($55,000/\text{mm}^3$) by heterophilia ($25,300/\text{mm}^3$), lymphocytosis ($23,100/\text{mm}^3$), and moncytosis ($5,500/\text{mm}^3$), besides a considerable increase in AST (88 U/L). All other values were within normal ranges (Bryant et al., 2012; Klaphake et al., 2017). The culture and antibiogram results were released after the animal's death, suggesting *Pseudomonas* spp. with resistance to the drugs amoxicillin associated with clavulanic acid, azithromycin, cephalexin, ceftiofur, ciprofloxacin, enrofloxacin, gentamicin, polymyxin B, and tobramycin, with sensitivity only to doxycycline.

3. Results and Discussion

Management errors such as inadequate temperature, humidity and ventilation, or other factors such as stress, reproductive seasons, and concomitant diseases may predispose to the occurrence of infections due to the proliferation of microorganisms (Ferreira et al., 2012, 2014, Martins et al., 2017, Sala et al., 2019). Because species of the genus *Pseudomonas* are part of the normal microbiota of captive snakes, generally there is a clinical disease when there is some factor that leads to immunosuppression, as in this case where the recent transport and change of environment may have contributed to the stress and consequent drop in immunity.

Infectious dermatitis, often manifested as cutaneous vesicles or fluid-filled "blisters" that can range from a slimy to caseous consistency, is a common infection in snakes, although under-reported. Many organisms, among fungi and bacteria, can be associated with these lesions, where *Pseudomonas* spp. stands out due to its high level of pathogenicity, which can progress to ulcers, fibrosis, and septicemia, often leading to animal death (Branch et al., 1998). Although reports of infections caused by *Pseudomonas* sp. are sporadically reported, vesicular dermatitis has been reported in few animals, as well as in *Micrurus corallinus* (Serrapicos, 2005), *Eunectes murinus* (Ferreira et al., 2014), and *Python molurus* (Branch et al., 1998), this being the first case of this type of dermatitis in *Morelia spilota* to date.

This bacterium is naturally very resistant to several antimicrobial agents, including most β -lactams, the older quinolones, chloramphenicol, tetracycline, macrolides, sulfamethoxazole-trimethoprim, and andrifampicin. Diagnosis is reached according to the patient's clinical condition, isolation, and identification of the etiologic agent, and as initial treatment, until the culture and antibiogram results, the use of broad-spectrum antibiotics is recommended. Aminoglycosides, such as gentamicin, amikacin, streptomycin, and tobramycin are the antibiotics of choice against *Pseudomonas* spp. (Ferreira et al., 2012, 2014; Martins et al., 2021); however, resistance was observed in this case, thus limiting their use. In the case reported here, the antibiotic chosen was enrofloxacin, besides the use of potassium permanganate baths, the latter being an antiseptic that acts against both bacteria and fungi and can be used as an adjuvant in infectious dermatitis treatments. Although there are few reports on potassium permanganate use, it is possible that it was responsible for the patient's clinical improvement, since the bacteria was resistant to enrofloxacin. However, it is suggested that the patient's death may have been caused by systemic involvement.

In reptiles, the WBC may vary according to numerous physiological factors, hindering the interpretation of this test, including stress, season, temperature, sex, age, and reproductive stage. However, the increased presence of leukocytes in peripheral blood may also be associated with inflammation caused by infection, neoplasia, or trauma. This pathological leukocytosis is often seen with deviations in leukocyte cell percentages, and heterophilia, lymphocytosis, and moncytosis can be found in chronic inflammatory responses to bacterial infections. Although azurophils are within normal values, it is known

that azurophilia in snakes is related to acute inflammatory lesions. The changes observed in this case, especially the presence of a considerable increase in the absolute amount of monocytes, heterophils, and lymphocytes, besides the normal amount of azurophils, indicate the presence of a chronic bacterial infection. (Jacobson et al., 1997; Stacy et al., 2011).

The clinical follow-up, the monitoring of environmental parameters such as temperature, humidity, and ventilation, the adequate feeding and sanitary management, as well as the environmental enrichment and the minimization of stress in captivity are essential for prevention and control of opportunistic infections, since they represent a risk for the animals as well as for the keepers and technicians who manage them (Ferreira et al., 2014).

4. Conclusion

This paper reports the occurrence of multidrug-resistant *Pseudomonas* sp. in *M. spilota*, presenting the clinical manifestation characterized by vesicular dermatitis, besides the microbiological, pathological, and therapeutic description of the case, thus expanding the data and helping in the medicine and ex situ conservation of snakes. Based on this study, new studies can be conducted to investigate the consequences that these animals may suffer in the wild and in captivity, and how this disease interferes with their longevity and well-being. Finally, this report indicates the need for further studies in order to establish the zoonotic potential of this infection and understand the importance of reptiles in the maintenance of this disease in humans.

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