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INFECTIOUS STOMATITIS IN CAPTIVE *SALVATOR MERIANAE* LIZARDS

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Abstract

Infectious stomatitis is a common ailment in captive reptiles, it arises from a combination of predisposing factors such as poor nutritional conditions, habitat issues or stress, where normal oral flora microorganisms act as pathogens, facilitating the disease development. This study aims to describe infectious stomatitis in a captive population of adult *Salvator merianae* lizards and to propose prophylactic measures for their maintenance in captivity. Within a population of 57 animals, a morbidity rate of 19.2% and a mortality rate of 7% were estimated. Microbiological analysis of oral mucosa revealed *Pseudomonas aeruginosa* susceptible to ceftazidime, ciprofloxacin, gentamicin, and amikacin. Symptoms ranged from asymptomatic to animals with mild oral cavity lesions or severe stomatitis, with some cases exhibiting respiratory complications. Histopathological examination of lung samples was consistent with caseous pneumonia. Intramuscular ceftazidime treatment and oral disinfection yielded excellent results for lizards with stomatitis, although a favorable response was not observed in animals with pneumonia.

Key words: Black and white tegu, caseous pneumonia, oral diseases, *Pseudomonas aeruginosa*, reptiles

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INFEKTIVNI STOMATITIS KOD GUŠTERA VRSTE *SALVATOR MERIANAE* KOJI SE DRŽE U ZATOČENIŠTVU

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Kratak sadržaj

Infektivni stomatitis je česta bolest kod gmizavaca koji se drže u zatočeništvu. Uzrokuje ga kombinacija predisponirajućih faktora kao što su loša ishrana, problemi sa staništem ili stres, gde normalni mikroorganizmi oralne flore deluju kao patogeni, što potpomaže razvoj bolesti. Ova studija ima za cilj da opiše infektivni stomatitis u populaciji odraslih guštera Salvator merianae koji se drže u zatočeništvu i da izloži predloge profilaktičkih mera za njihovo držanje u zatočeništvu. U okviru populacije od 57 životinja utvrđena je stopa morbiditeta od 19,2% i mortaliteta od 7%. Mikrobiološka analiza oralne sluzokože pokazala je prisustvo *Pseudomonas aeruginosa* koji je osetljiv na ceftazidim, ciprofloksacin, gentamicin i amikacin. Simptomi su bili raznoliki - od asimptomatskih životinja do onih koje su ispoljile simptome teškog stomatitisa, blage lezije usne duplje, a neke su imale i respiratorne komplikacije. Histopatološki pregled uzoraka pluća ukazao je na kazeoznu pneumoniju. Intramuskularni tretman ceftazidimom i oralna dezinfekcija dali su odlične rezultate za guštere sa stomatitisom. Međutim, nije primećen povoljan odgovor kod životinja sa pneumonijom.

Ključne reči: Crni i beli tegu, kazeozna pneumonija, oralne bolesti, *Pseudomonas aeruginosa*, reptili

INTRODUCTION

Adequate zootechnical management stands out as the most crucial factor for maintaining healthy reptiles bred in captivity. Inappropriate breeding conditions, such as overcrowding, nutritional deficiencies, infections, and parasitosis lead to stress-induced immunosuppression (Cobos and Ribas 1987; Meredith and Redrobe, 2012; Zhou et al., 2020; Tian et al., 2022). Numerous microorganisms constitute oral microbiota in reptiles. Nevertheless, disrup-

tions in the homeostasis of the immune response caused by the captive environment transform the commensal oral microbiota into opportunistic pathogens (Grego et al., 2017; Vega-Manriquez et al., 2018).

Infectious stomatitis, or "mouth rots," is a common oral mucosa infection in reptiles bred in captivity. While this disease has been predominantly described in snakes (Peñuela Gomez and Brieva Rico, 2007; Rojas-Sereno et al., 2015; Martins et al., 2021), it also affects turtles and some lizard species (Herrera Ramírez, 2008; Pereira et al., 2021). Symptoms of this pathology include sialorrhea, petechiae, plaques around the lips and mouth, facial malformations, gingival abscesses, and teeth loss. In cases that are not promptly controlled, the exudate from ulcerative stomatitis may be swallowed or aspirated, leading to gastroenteritis or bacterial pneumonia. In severe cases, the pathogen may also enter the general circulation, causing septicemia and death (Cobos and Ribas 1987; Mader, 2006; Meredith and Redrobe, 2012; Rodríguez Molano, 2015; Pereira et al., 2021; Rojas-Sereno et al., 2015; Doneley et al., 2018).

In saurians, pathological processes tend to be multifactorial, slow-progressing, and challenging to diagnose due to their inherent resistance and ability to mask symptoms. For this reason, in most cases, diseases are detected at advanced stages, which complicates the success of treatment and, in the case of captive populations, epidemiological control (Mader, 2006; Meredith and Redrobe, 2012).

This study provides a detailed report on the clinical manifestations, lesions, and treatment in a captive population of *Salvator merianae* lizards with infectious stomatitis.

MATERIALS AND METHODS

Animals

The study included 57 adult individuals from the *Salvator merianae* lizard breeding facility at the Facultad de Agronomía, Zootecnia y Veterinaria of the Universidad Nacional de Tucumán, province of Tucumán, Argentina (26° 51'S and 65° 17'W). The animals were housed in open-air enclosures with masonry fences, equipped with shelters containing dry grass and shade. For the welfare of adult *Salvator merianae* individuals, the minimum living space of 2 m^2, was provided (Manes, 2016). Ad-libitum feeding consisted of a diet specifically designed for this captive-bred species (Vega Parry and Manes, 2000). For individual health monitoring, radiofrequency identification devices (micro transponder ID-100, Trovan Electronic Identification, Rosenbusch, Buenos Aires, Argentina) were used.

All experiments, including all animal handling protocols, were carried out in accordance with the Principles of Laboratory Animal Care (National Institutes of Health, publication N° 85- 23, revised 1985), as well as specific national laws. All experiments were carried out and approved by the Ethics Committee of Consejo de Investigaciones de Universidad Nacional de Tucumán (CIUNT).

Characterization of pathogens

The samples from the buccal and gingival mucosa of animals exhibiting signs of advanced stomatitis were collected using swabs and transported and preserved in Stuart medium. Bacteriological culture and antibiogram testing were conducted using the agar diffusion method (Kirby-Bauer test).

Histopathology

Histopathological analysis of lung biopsies from recently deceased animals was performed using standard procedures, including fixation with 10% neutral buffered formalin, embedding, sectioning, and hematoxylin and eosin staining (Suvarna et al., 2018).

RESULTS

Epidemiology and clinical signs

The disease affected both males and females. Out of a total number of 57 adult animals that were examined, 4 exhibited symptoms of advanced stomatitis, 7 showed signs of mild stomatitis, and another 4 were cases with respiratory and/or systemic complications. The remaining 42 animals did not show any lesions in the oral mucosa indicative of clinical stomatitis. As population indicators, the morbidity rate of stomatitis with clinical signs was estimated at 19.2%, with a mortality rate of 7%.

The 4 individuals with the signs of advanced stomatitis had petechiae and ecchymosis in the oral mucosa, periodontitis, they experienced loss of dental pieces, and granulomatous plaques in the oral and lingual mucosa. In some cases, these signs were accompanied by oral, ocular, or nasal discharge (Figure 1A).

The seven animals that manifested mild signs of stomatitis, had inflammation of the oral and gingival mucosa, but without hemorrhagic lesions or any type of plaques.

The 4 animals with severe stomatitis and caseous necrosis in the mouth and tongue died due to respiratory and systemic complications. Only two of them showed evident signs of respiratory distress with dyspnea, white foamy expectorations, and loss of appetite. The other 2 did not show evident signs of respiratory complications, and the diagnosis was confirmed through post-mortem lesions (Figure 1B).

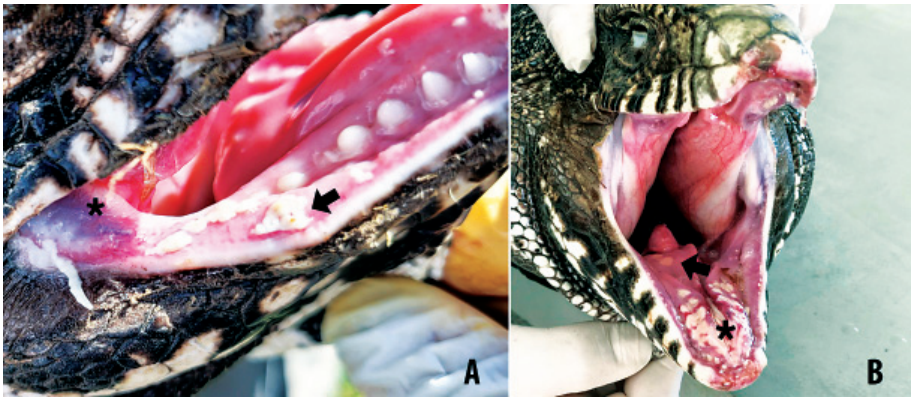

Figure 1. A. Adult female *S. merianae* lizard with stomatitis; arrow indicates granulomatous plaque on oral mucosa, asterisk indicates inflamed, congested, and ecchymotic area at oral commissure. B. Adult male *S. merianae* lizard with severe stomatitis; asterisk indicates apical region of the tongue and oral mucosa with caseous necrosis, arrow indicates granulomatous plaque on the body of the tongue.

Macroscopic findings

The necropsy of the recently deceased lizards revealed that the most affected organs were the lungs, although some macroscopic lesions were also found in the mouth, the fat bodies, and the liver. The fat bodies were notably congested and friable, with hemorrhagic lesions. Additionally, the friable liver had changed in its color. The lungs were the most affected organs, significantly hyperemic, and hemorrhagic with numerous pinpoint granulomatous plaques similar to those found in the oral cavity (Figure 2).

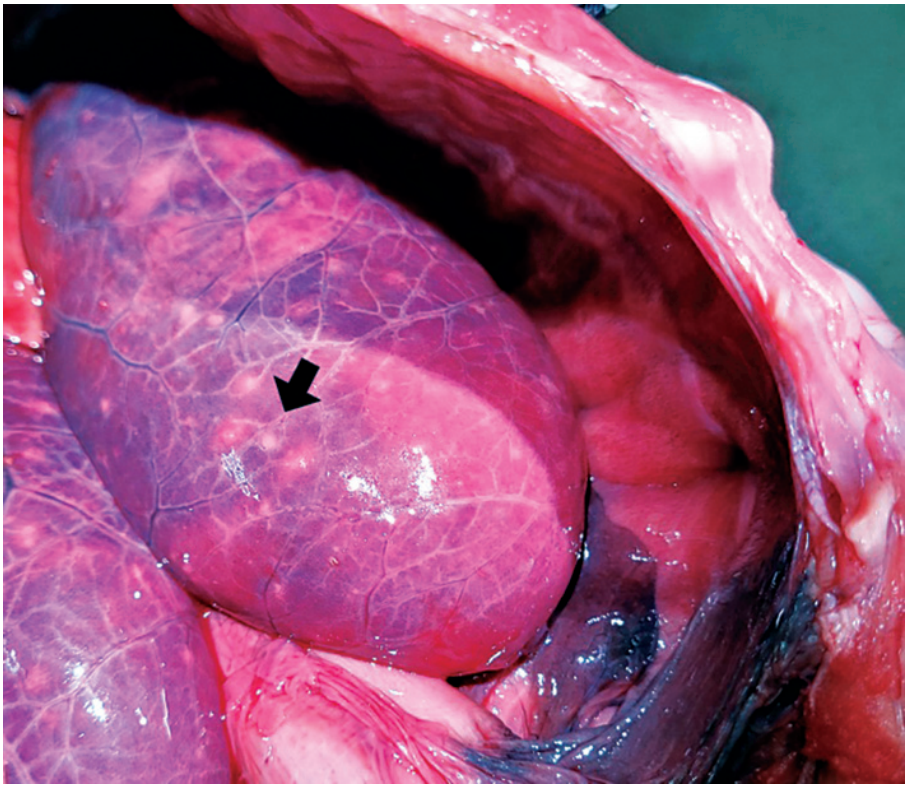

Figure 2. Lungs with numerous small granulomatous lesions (arrow).

Histopathology findings

In the submesothelial layer of the visceral serous tunic and the stroma of type III trabeculae in both lungs, inflammatory hyperplasia, characterized by the profusion of collagen fibers, was evident. Moreover, in the lumen of the pulmonary venules, there was a notable abundance of lymphocytes (Figure 3A). Necrotic lesions with infiltration of the pulmonary parenchyma and eosinophilic areas centrally located to the granulomatous lesion, composed of distinctive amorphous tissue of caseous necrosis, were also prominent (Figures 3B and 3C).

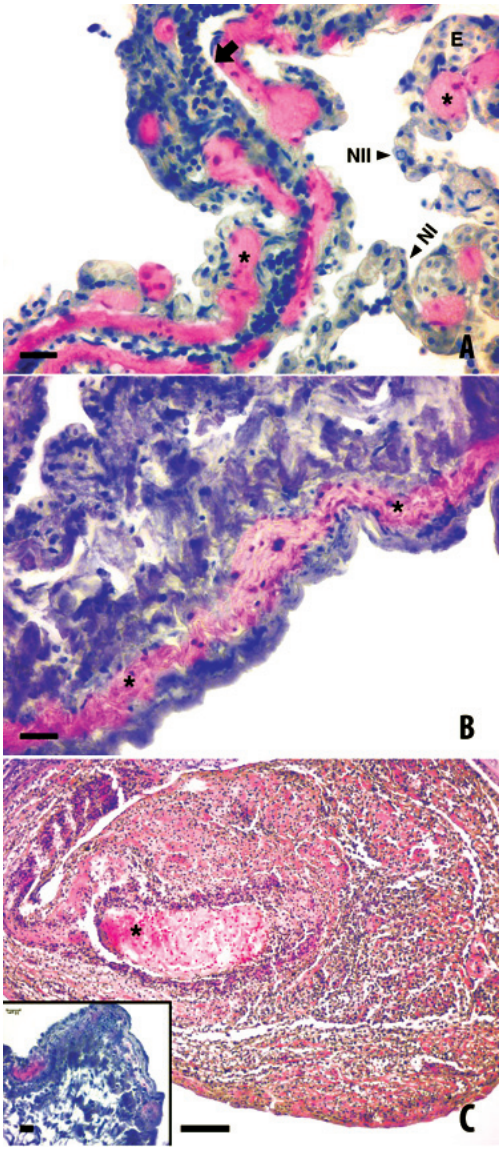

Figure 3. A. Detailed view of foveolar epithelium with marked hypertrophy of type III trabeculae (asterisks). Note the abundance of lymphocytes (arrow). NI, type I pneumocytes; NII, type II pneumocytes; E, erythrocytes. Scale bar 10 μm. B. Detailed view of visceral serous layer of the lung. Note the abundance of collagen fibers in the submesothelial layer (asterisks). Scale bar 10 μm. C. Lung section with granulomatous lesion (asterisk). Scale bar 50 μm. The insert shows characteristic necrotic tissue with absence of cellular boundaries and pyknotic nuclei. Scale bar 10 μm.

Culturing and antibiogram

Bacteriological culture of the oral mucosa of animals with the clinical disease revealed few Gram-positive cocci and few Gram-negative bacilli, as well as polymorphic nuclear cells. *Pseudomonas aeruginosa*, sensitive to antibiotics such as ceftazidime, ciprofloxacin, gentamicin, and amikacin, was isolated.

Treatment and evolution of animals with clinical signs

Based on the antibiogram results, intrinsic characteristics of each antibiotic (therapeutic margin, toxicity, duration of action, ease of application), and cost analysis, ceftazidime antibiotic therapy was chosen.

Animals with the signs of stomatitis with evident oral mucosa plaques and lesions underwent the following treatment: cleaning of wounds with 10 vol. hydrogen peroxide or 0.05% chlorhexidine and manual removal of oral plaques. Simultaneously, they were supplemented with 5000 IU of oral vitamin A once a week.

Depending on the characteristics of each patient and the severity of the clinical picture, between 3 and 6 applications of intramuscular ceftazidime at 30 mg/kg were performed every 72 hours until the reversal of symptoms.

In cases of mild or advanced stomatitis without respiratory complications, the treatment was highly effective, and the patient's evolution was favorable (Figure 4). However, in animals with clinical signs of pneumonia, the treatment was not effective.

Figure 4. Evolution of a stomatitis case: A and B. Initial state of oral wounds, left and right profiles, respectively. C. Oral wounds at 2 weeks after the start of treatment. Arrows indicate granulomatous plaques at both commissures, asterisk indicates petechiae. D. Resolution of oral wounds and patient's discharge 6 weeks after treatment initiation.

Population Management Measures

For metaphylactic treatment, the entire breeding stock received a single dose of 30 mg/kg of intramuscular ceftazidime. Weekly controls of the oral mucosa and body condition of 10 randomly selected animals were conducted for a month. When an individual showed any signs or suspected symptoms of stomatitis, it was isolated to start antibiotic treatment, vitamin A supplementation, and cleaning and disinfecting of the oral mucosa.

After a month, and in order to avoid stress from manipulation, inspections consisted of visual examination of animals in the corral, and only if suspicious, the animal was restrained for mucous membrane inspection. An animal was considered suspicious if it exhibited at least one of the following symptoms:

oral, ocular, or nasal discharge of any type, weight loss of body condition, or dehydration.

Sick or suspected animals were separated from the rest in an isolation area with the same shade, shelter, water, and ration conditions as the rest of the enclosures. Feeders, drinkers, and shelters throughout the breeding facility were disinfected with 0.1% sodium hypochlorite, and the substrate in all shelters was renewed.

DISCUSSION

The oral and intestinal microbiota varies significantly among different groups of reptiles and is influenced by their habitat, physiology, and nutrition. Generally, the oral flora in saurians is primarily composed of Gram-negative bacteria, such as *Escherichia coli, Salmonella, Pseudomonas, Proteus,* and *Aeromonas* (Meredith and Redrobe, 2012; Tian et al., 2020, 2022). These bacteria can be isolated from both healthy and diseased animals. It is known that healthy reptiles can transmit *Salmonella* or *P. aeruginosa*, representing a major zoonotic risk associated with keeping reptiles (Meredith and Redrobe, 2012; Martins et al., 2021). This is the first study reporting *P. aeruginosa* as a potential opportunistic pathogen causing infectious stomatitis in *S. merianae.*

The clinical signs observed in our animals coincided with those reported for stomatitis in other captive reptiles (Cobos and Ribas 1987; Mader, 2006; Meredith and Redrobe, 2012; Rojas-Sereno, 2015; Hedley, 2016; Doneley et al., 2018; Pereira, 2021). The lesions found in the oral cavity of diseased animals were similar to those described for *Boa constrictor amarali* with caseous stomatitis caused by *P. aeruginosa* (Martins et al., 2021). In reptiles, pneumonia signs often appear late, when the infection is chronic with significant respiratory involvement (Mader, 2006; Rodríguez Molano, 2015). In most cases observed in this study, the condition was limited to oral infection. However, in animals with pneumonia, macroscopic and histopathological lesions were consistent with chronic inflammatory processes, characterized by a marked increase in collagen fibers and notable lymphocytic infiltration. Timely treatment with intramuscular ceftazidime every 72 hours and oral antiseptics proved to be the appropriate combination for treating stomatitis in *S. merianae*. The combination of clinical examination, oral culture and antibiogram, histopathology, and favorable response to treatment, enabled us to confirm the diagnosis without the need for more expensive complementary tests.

The incidence of bacterial diseases in captive lizards is often associated with a compromised immune system resulting from overcrowding, trauma, nutritional deficiencies, infections, and excessively low temperatures that can

predispose normal microbial flora to act as opportunistic pathogens (Mader, 2006; Hedley, 2016; Van Zanten and Simpson, 2021). Regarding predisposing factors, overcrowding and poor management as promoters of stomatitis were ruled out. Indeed, we have optimized the zootechnical management of captive *S. merianae*, as well as the breeding conditions in which these lizards are kept and reproduced. This includes spacious enclosures with over 2 m^2/animal, shelters resembling their natural habitat, dirt floors for digging, and other elements of environmental enrichment (Manes, 2016; Van Zanten and Simpson, 2021). Although the animals are fed a diet specifically designed for this species (Vega Parry and Manes, 2000), maintaining a sustained mono diet for approximately 20 years, it is likely that they require supplementation of vitamins A, D, and E. Vitamin A deficiencies have been linked to stomatitis and inflammation of the upper respiratory tract (Herrera Ramírez, 2008; Rodríguez Molano, 2015). In this study, oral administration of vitamin A was chosen to coincide with the oral inspection of each patient, avoiding additional intramuscular injections that could be painful and stressful for the animal. Stress from increased frequency or duration of handling, including health management, predisposes the animals to illness and complicates the healing process (Doneley et al., 2018).

The immune response of reptiles is subjected to seasonal temperature-dependent alterations, with a maximum response observed when they are maintained near their preferred optimal body temperature (Mader, 2006; Doneley et al., 2018). Prolonged maintenance of reptiles at temperatures ranging from 15 °C to 21 °C predisposes them to opportunistic diseases. The innate and adaptive arms of the reptilian immune system are accepted to function optimally at the preferred body temperature of the animal. Many reptiles are kept at suboptimal temperatures, and simply warming them up can lead to significant improvements in their immunological defenses (Doneley et al., 2018). The monthly average temperature recorded in the region, where the breeding facility is located during the brumation period (May to September), was of 14.8 °C between 2018 and 2022 (EEAOC, 2023). This temperature would be at the limit of the suggested optimum for normal brumation (3.8 °C to 15 °C). Our results suggest that untimely winters with inappropriate temperatures for proper brumation may be a predisposing factor for stomatitis in captive *S. merianae*.

There is little knowledge about the immune system of reptiles. Some agents of reptiles are more or less infectious, but for most of them, the level of infectivity is unknown (Doneley et al., 2018). In our study, it is likely that *P. aeruginosa* acted as an opportunistic agent, and the great diversity of clinical

manifestations observed in our population may be attributed to the intrinsic immunity of each animal. The physiological state of each individual can impact susceptibility to diseases, even under similar captive conditions. Gravid females, males during breeding season, dominance of food sources, or other resources by enclosure mates, together with the effects of hierarchical stress, may predispose to illness (Meredith and Redrobe, 2012; Doneley et al., 2018).

CONCLUSIONS

This article provides a detailed report of clinical manifestations and macroscopic and histopathological findings in a captive population of *S. merianae* lizards with stomatitis. Identification of each individual facilitated precise treatment monitoring and the evolution of each patient. Furthermore, periodic follow-ups within the population have allowed us to suggest sanitary management measures to reduce the prevalence of this disease in captive animals.

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Author's Contributions

OLSL: Animal management, diagnosis and treatment of patients, drafting the manuscript. FHCC: Histopathological analysis, drafting the manuscript.

Competing interests

The author(s) declare that they have no competing interests.

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