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Sepsis by *Plesiomonas shigelloides*, *Citrobacter freundii* and *Aeromonas jandaei* in a green iguana (*Iguana iguana*)[1]

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ABSTRACT.- Paranhos G.F., Oliveira Filho H.S., Duarte J.L.C, Oliveira R.L., Lima E.S., Fernandes A.C.C., Firmino M.O., Silva W.I. & Araújo J.L. 2024. **Sepsis by *Plesiomonas shigelloides*, *Citrobacter freundii* and *Aeromonas jandaei* in a green iguana (*Iguana iguana*).** *Pesquisa Veterinária Brasileira 44:e07432, 2024*. Departamento de Ciências Veterinárias, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Rodovia 12, Areia, PB 58397-000, Brazil. E-mail: lealjeann@gmail.com

Iguanids are susceptible to several bacterial infections, especially those caused by Gram-negative bacteria. The development of these diseases in reptiles is related to management, inadequate sanitary conditions, and immunosuppression. This study aims to describe the anatomopathological and microbiological aspects of a case of co-infection by *Plesiomonas shigelloides*, *Citrobacter freundii* and *Aeromonas jandaei* diagnosed in a free-living green iguana (*Iguana iguana*). Macroscopically, the lesions were mainly located in skeletal muscle, myocardium, small intestine, and liver, characterized by white-yellowish, multifocal, friable, irregular areas associated with necrosis and hemorrhage. In the histopathological analysis, basophilic bacillary structures corresponding to bacterial aggregates were observed in the skeletal muscle, myocardium, hepatic parenchyma, kidney, stomach and small intestine associated with areas of thrombosis, necrosis and hemorrhage. The diagnosis of sepsis by *P. shigelloides*, *C. freundii* and *A. jandaei* was confirmed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) associated with the anatomopathological and microbiological findings observed in this case.

INDEX TERMS: Bacterial coinfection, Iguanidae, reptile, septicemia.

RESUMO.- [**Sepse por *Plesiomonas shigelloides*, *Citrobacter freundii* e *Aeromonas jandaei* em uma Iguana verde (*Iguana iguana*).**] Iguanídeos são suscetíveis a diversas infecções bacterianas, principalmente aquelas causadas por bactérias Gram-negativas. O desenvolvimento destas enfermidades em répteis está relacionado a manejos, condições sanitárias inadequadas e imunossupressão. Este estudo tem por objetivo descrever os aspectos anatomopatológicos e microbiológicos de um caso de coinfecção por *Plesiomonas shigelloides*, *Citrobacter freundii* e *Aeromonas jandaei* diagnosticado em uma iguana verde de vida livre (*Iguana iguana*). Macroscopicamente, as lesões localizavam-se principalmente em musculatura esquelética, miocárdio, duodeno e fígado, caracterizando-se por áreas branco-amareladas, multifocais, friáveis, e irregulares associadas à necrose e hemorragia. No histopatológico foram vistas estruturas bacilares basofílicas correspondentes a agregados bacterianos em músculo esquelético, miocárdio, parênquima hepático, rim, estômago e intestino delgado associados a áreas de trombose, necrose e hemorragia. O diagnóstico de sepse por *P. shigelloides*, *C. freundii* e *A. jandaei* foi confirmado pela técnica de ionização por dessorção a laser assistida por matriz com analisador por tempo de voo (MALDI-TOF) associada aos achados anatomopatológicos e microbiológicos observados neste caso.

TERMOS DE INDEXAÇÃO: Coinfecção bacteriana, Iguanidae, répteis, septicemia.

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INTRODUCTION

Iguanas are arboreal lizards belonging to the family Iguanidae. The genus *Iguana* comprises thirty species, with the green iguana (*Iguana iguana*) being the most widespread in the Americas (Phillips 1990). In Brazil, green iguanas are easily found in the Amazon, Pantanal and Caatinga biomes (Campos & Desbiez 2013).

Iguanids are susceptible to numerous infectious diseases, including infections caused by Gram-negative bacteria of the genera *Aeromonas, Citrobacter, Salmonella, Pseudomonas* and *Escherichia*. These agents become more pathogenic under immunosuppressive host conditions or when these bacteria are resistant to antibiotics (Jacobson 2007). The development of bacterial infections in captive wildlife is often related to inadequate management and improper sanitary conditions of the enclosure, mainly related to temperature and humidity (Chinnadurai & Devoe 2009), but can also occur in free-living animals (Daskin & Alford 2012) due to anthropological actions, such as deforestation, hunting, habitat fragmentation and trafficking (Wolfe et al. 2005).

The present study aims to report the pathological and microbiological aspects of a case of co-infection by *Plesiomonas shigelloides*, *Citrobacter freundii* and *Aeromonas jandaei* diagnosed in a free-living green iguana (*I. iguana*).

MATERIALS AND METHODS

Ethical approval. This study was conducted following the guidelines proposed by the University Institutional Ethics on Animal Use Committee (CEUA) of the "Universidade Federal da Paraíba" (UFPB), which approved the use of the tissues for teaching and research for patients upon submission for necropsy, protocol number: 9756290419.

A green iguana was referred to the Veterinary Hospital (HUV) of the UFPB, Areia, Paraíba with a history of trauma and absence of pelvic limbs and tail movements after a motor vehicle collision. Initially, the animal was submitted to clinical evaluation and complementary exams, such as full body radiography with laterolateral and dorsoventral projections, urinalysis and surgical treatment through an enterotomy.

Necropsy and microscopic examinations were routinely performed at the Veterinary Pathology Laboratory of HUV-UFPB. All organs were collected, fixed in 10% formalin, trimmed, and sent for routine histopathological processing. Slides were cut at 5μm and stained by hematoxylin and eosin (HE).

Heart fragments and intestinal swabs were collected in a Stuart medium and sent for microbiological isolation. The samples were first plated in 5% defibrinated sheep blood agar and MacConkey agar, later being also plated in Hektoen agar, xylose lysine deoxycholate agar (XLD) and *Salmonella-Shigella* agar. All colonies grew at 37°C for 24 hours.

Subsequently, Petri dishes with brain and heart infusion (BHI) agar medium were sent to the Milk Quality Research Laboratory at the "Faculdade de Medicina Veterinária e Zootecnia" (College of Veterinary Medicine and Animal Science - FMVZ) at the "Universidade de São Paulo" (USP) for matrix-assisted laser desorption/ionization time of flight (MALDI-TOF), a more selective method for the confirmation of agents. In MALDI-TOF, biochemical tests were also performed, such as triple sugar iron (TSI), sulfide indole motility (SIM), motility indole ornithine (MIO), citrate, urea, Voges Proskauer (VP), methyl red (VM), phenylalanine, catalase and oxidase.

RESULTS

On clinical examination, the patient presented mild dehydration and flaccid paralysis of the pelvic limbs and tail, with no signs of superficial pain reflexes. On the radiographic examination, a radiopaque area was evidenced in the intestinal loops, which was suggestive of a fecaloma. In view of this finding, we chose to perform surgical treatment through enterotomy.

During enterotomy, a fecaloma was confirmed at the final third of the rectum, and adherence of the urinary bladder to the duodenum was seen. In addition, the urinary bladder was hemorrhagic and a transurgical cystocentesis was performed.

On urinalysis, the urine sample was macroscopically reddish and cloudy, which corresponded to hematuria. Microscopically, the sample presented numerous erythrocytes in addition to thrombocytes and leukocytes.

After the surgical procedure, the animal, weighing 2.7kg, was hospitalized and received anti-inflammatory (Ketoprofen; 0.05mL; IM; SID), analgesic (Tramadol; 0.4mL; IM; SID) and antibiotic therapy (Enrofloxacin; 0.5mL; IM; SID). Two weeks after surgery, the animal died and was referred to necroscopic examination.

During necropsy, a focally extensive yellowish area was observed in the skeletal muscle of the left pelvic limb. Multifocal areas of hemorrhage in the contralateral limb were also noted (Fig.1). There were multifocal punctiform white-yellowish areas on the epicardial surface in the apex region of the heart (Fig.2). Additionally, on the cut surface, a focally extensive irregular yellow area was seen in the myocardium (Fig.3). In the duodenum, multifocal, yellowish-white, irregular, transmural areas were identified (Fig.4). The urinary bladder was filled with a cloudy fluid and had multifocal areas of congestion and hemorrhage in the mucosa and vesicular serosa. No significant gross lesions were observed in the kidneys, brain and other organs.

On histopathology, degenerative and necrotic changes of the myocytes of the pelvic limb, consisting of ruptured myofibrils associated with hemorrhage and fibrin that separate the skeletal muscle fibers were observed (Fig.5). In the myocardium, the fibers were fragmented, shrunk and separated by multifocal areas of necrosis associated with basophilic bacillary structures arranged in clusters (Fig.6). In the hepatic parenchyma there were multifocal to coalescing areas with fibrin deposition, multiple degenerated and necrotic hepatocytes associated with the presence of basophilic granular structures phagocytosed by melanomacrophages (Fig.7). The gastric mucosa and submucosa were expanded by thrombi containing bacterial myriads and fibrin that obstructed the capillaries, accompanied by erosion, ulceration and hemorrhages in the epithelial surface (Fig.8). In the kidneys, the same basophilic bacterial myriads were seen distributed multifocally throughout the parenchyma, affecting vessels and glomeruli (Fig.9 and 10).

Microbiological and proteomic findings

In the *Salmonella-Shigella* agar, two types of colonies grew from the samples collected from the intestine, and one grew from samples collected from the heart. The first colony type of the intestine presented a rounded, medium-sized, shiny, pink appearance composed of lactose fermenting (Lac+) bacteria. The second colony of the intestine and the isolate of the heart were irregular, mucoid, shiny and yellowish in appearance and comprised of non-lactose-fermenting bacteria (Lac-), suggesting *Shigella* spp. Fragments plated on XLD agar, Hektoen agar, and 5% defibrinated sheep blood agar showed no bacterial growth.

Biochemical tests showed that these isolates were non-glucose-fermenting bacteria, negative for citrate, malonate, phenylalanine, Voger-Proskauer, gelatin, H_2S production, indole, urease and motility tests, and positive for nitrate, catalase, and methyl red tests. Additionally, MALDI-TOF was used for final confirmation of the bacterial isolates and the following bacteria were identified: *Aeromonas jandaei* (heart), *Plesiomonas shigelloides* (intestine) and *Citrobacter freundii* (intestine).

DISCUSSION

The diagnosis of sepsis by *Plesiomonas shigelloides*, *Citrobacter freundii* and *Aeromonas jandaei* in the case presented in this study was established based on anatomopathological and

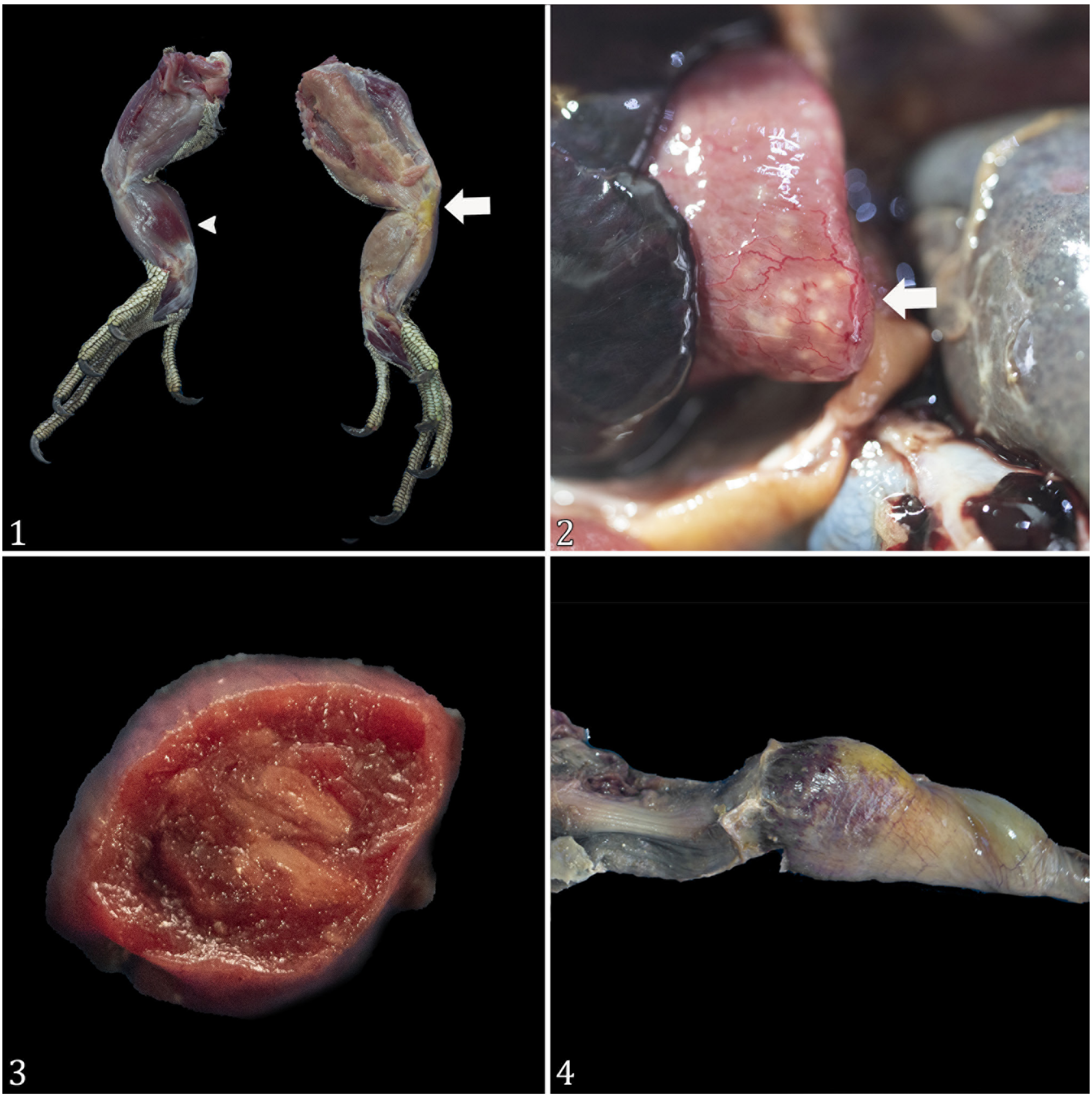

Fig.1-4. (**1**) Focally extensive yellowish area on the left pelvic limb (arrow) with severe multifocal hemorrhages (arrowhead) in skeletal musculature. (**2**) White-yellowish punctiform areas are observed on the epicardial surface (arrow). (**3**) Focally extensive area of yellow-brownish, friable, irregular surface in the myocardium. (**4**) White-yellowish multifocally areas in the intestinal serosa of duodenum. At the cut, the lesions had a transmural aspect.

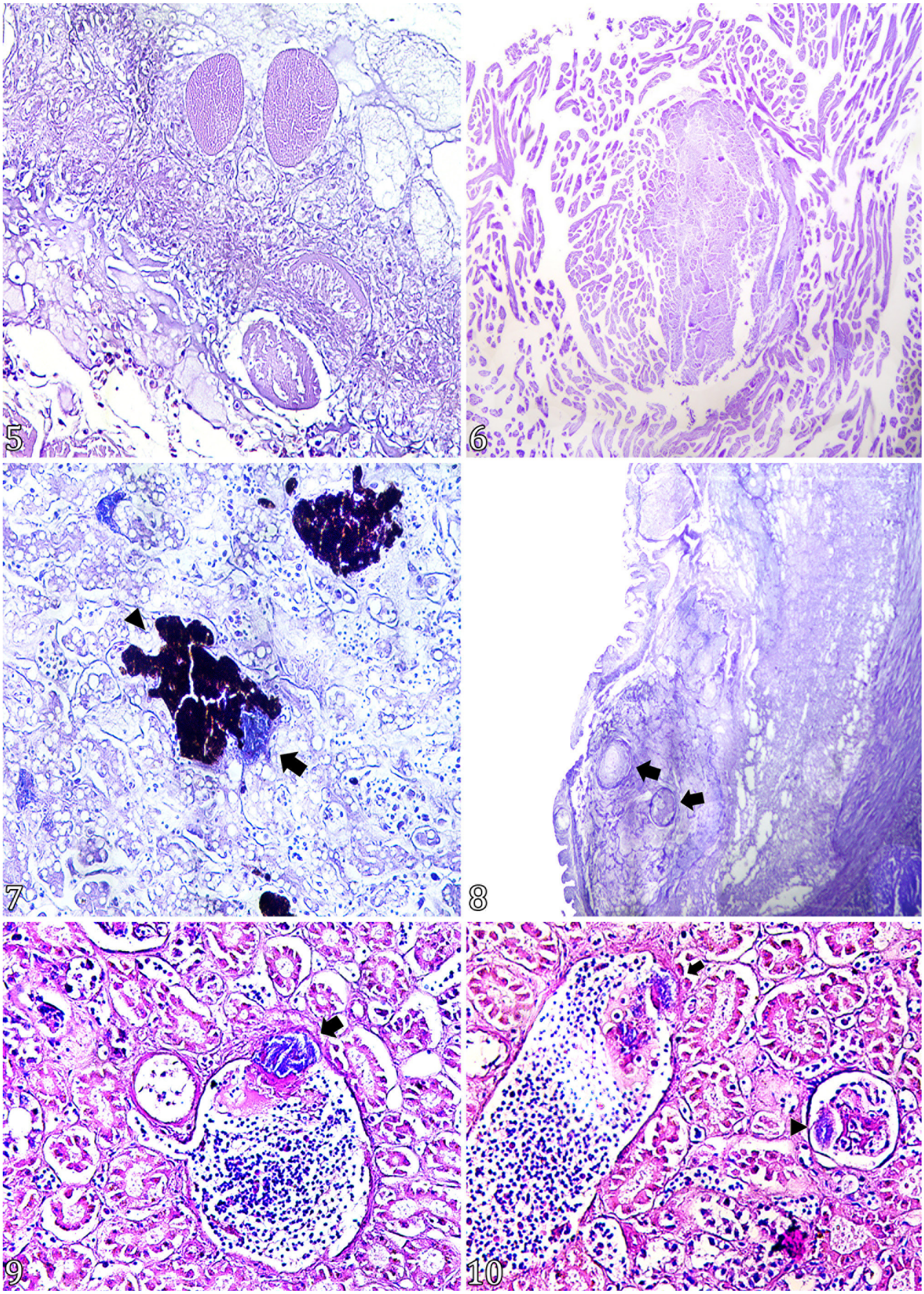

Fig.5-10. (**5**) Multifocal areas of myofiber degeneration and a focally extensive area of necrosis in skeletal musculature. HE, obj.40x. (**6**) Multifocal bacterial colonies associated with areas of myocardial necrosis. HE, obj.20x. (**7**) Melanomacrophage centers (arrowhead) phagocytosing a basophilic bacterial aggregate (arrow) in hepatic parenchyma. HE, obj.20x. (**8**) Multifocally, within the mucosa and submucosa, thrombi (arrow) associated with areas of necrosis and ulceration in the gastric epithelium. HE, obj.4x. (**9**) Basophilic bacillary structures arranged in aggregates present in capillaries (arrow). HE, obj.20x. (**10**) Multifocal areas with the presence of basophilic bacterial myriads in the blood vessels (arrowhead) and Bowman's space (arrowhead). HE, obj.20x.

microbiological findings, which were later confirmed by MALDI-TOF analysis.

The reported trauma in this case may have created a potential gateway for opportunistic infections, as observed in other wild animals (Dai et al. 2011). In these lesions, particularly in domestic animals, there is a dysregulation of neutrophils, leading to a decrease in phagocytic function (Janicova & Relja 2021). This could be related to a possible dysfunction of heterophils in this iguana. The etiology of such traumas mainly includes compression or crushing, resulting in acute death due to hemorrhages, organ dysfunction, infections, and sepsis (Simpson et al. 2009), which aligns with the macroscopic findings described in this animal.

In a study carried out on dogs, sepsis was found to result in acute kidney injury with elevated serum creatinine levels in about 12% of cases (Keir & Kellum 2015), a finding not observed in the present case. In another study conducted on sheep, sepsis-induced reduced oxygen perfusion to the brain in the initial three hours and to the renal medulla within five hours (Okazaki et al. 2021). Despite the less pronounced kidney lesions and the absence of central nervous system alterations in this iguana, a similar mechanism may have contributed to the death of this reptile.

In a bacterial co-infection involving *P. shigelloides*, *Salmonella enterica* spp. *arizona*, and *Enterobacter cloacae* described in a Burmese python (*Python bivittatus*), factors such as anorexia, nutritional deficiency, and hypothermia may have induced the release of glucocorticoids that lead to immunosuppression and proliferation of bacteria with the release of endotoxins to several organs (Abba et al. 2016). In another report of septicemia caused by *Aeromonas hydrophila* in captive crocodiles (*Crocodylus johnstoni* and *Crocodylus porosus*), a thermoregulatory imbalance resulted in immunosuppression and bacterial dissemination, accompanied by the release of hemolysins and systemic enterotoxins (Roh et al. 2011). These processes may explain the pathogenesis of the lesions observed in the present study, although the pathophysiology of sepsis in reptiles remains poorly understood.

Another hypothesis to consider in the etiopathogenesis of this patient is that several bacteria, including *P. shigelloides* (Janda et al. 2016), *Aeromonas* spp. (Merino et al. 1995) and *Citrobacter* spp. (Samonis et al. 2009), are part of the normal intestinal microbiome in reptiles. However, under inadequate management, stress, and immunosuppression (Wellehan & Gunkel 2004), these bacteria can trigger disease, often remaining undiagnosed due to subclinical conditions.

Stress plays a crucial role in the dissemination of these pathogens and has been associated with outbreaks in zoo penguins (Nimmervoll et al. 2011), which may have also contributed to the possible immunosuppression in this green iguana since this species experiences constant stress during management (Kalliokoski et al. 2012). This factor may also have triggered a case of sepsis caused by *P. shigelloides* in a gray wolf (*Canis lupus*), where bacterial aggregates were observed in the gastrointestinal tract and liver parenchyma associated with areas of congestion and necrosis of these organs; lesions similar to those described in the present report (Kim et al. 2021).

The presence of septic thromboembolism associated with multifocal areas of hemorrhage, inflammation, necrosis, and the presence of basophilic granular structures in various organs indicates a sepsis process, as observed in a study conducted on Gila monsters (*Heloderma suspectum*), where 52% of the inflammatory diseases in these animals were caused by bacterial infections and 22% of them led to septic shock (Magnotti et al. 2021).

The congestive and hemorrhagic vascular disorders observed in the skeletal musculature and intestine, in this case, may be associated with the release of bacterial toxins, as described in sepsis caused by *A. jandaei* in Nile tilapia (*Oreochromis niloticus*) (Dong et al. 2017). Similar observations were made in systemic co-infections involving *A. jandaei* and *A. hydrophila* in freshwater crocodiles (*Crocodylus siamensis*), which were also associated with hepatomegaly and alveolar hemorrhages (Pu et al. 2019).

Citrobacter freundii also demonstrates high pathogenicity and lesions in the intestinal epithelial barriers due to inflammation, necrosis, and dysbiosis, allowing the bacteria to spread to the liver, causing lipid vacuolar degeneration and necrosis (Li et al. 2022). Transmural caseous lesions, as observed in the intestine of this iguana, have been described in a case of sepsis caused by *C. freundii* in loggerhead turtles (*Caretta carretta*), with the gallbladder and intestinal diverticulum being the most affected organs (Inurria et al. 2024).

The proteomic characterization of bacteria such as *P. shigelloides*, *A. jandaei* and *C. freundii* complements the description of the strains and allows a more accurate diagnosis (Kolínská et al. 2010, Mursalim et al. 2022, Ahmed et al. 2023), as well as in the case of co-infection of this iguana. In the identification of Gram-negative Enterobacteriaceae, this method demonstrated an efficacy of approximately 95% in the differentiation of peptides of these microorganisms (Pribil & Fenselau 2005, Barbosa et al. 2021), data similar to those described in this study. In the reported case, the collection and submission of samples for bacterial culture and the analysis of MALDI-TOF were essential to identify the etiologic agents involved and confirm the diagnosis of sepsis.

CONCLUSIONS

The comprehensive evaluation of macroscopic and microscopic findings in this study supports the presence of a systemic infection. However, the precise identification of the bacterial species involved was only achieved through a highly sensitive diagnostic method such as the MALDI-TOF.

The identification of these etiological agents holds great significance not only in understanding infections and their associated anatomopathological manifestations in reptiles but also in shedding light on similar occurrences in other wildlife and even humans. Therefore, it serves as a valuable tool in conservation medicine and public health, enabling informed decision-making and targeted interventions.

Authors' contributions.- Gabriel F. Paranhos perfomed the experimental design, collected and wrote the data for the manuscript with support from José Lucas C. Duarte, Hodias S. Oliveira Filho and Millena O. Firmino. Arthur C.C. Fernandes, Ewerton S. Lima and Welitânia I. Silva performed the microbiological diagnosis of the samples. Rafael L. Oliveira performed the clinical and surgical examination of the animal. Jeann L. Araújo developed the experimental design, verified the analytical methods, monitored and performed the critical review of the manuscript. All authors discussed the results and contributed to the final manuscript.

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Conflict of interest statement.- The author declares that there are no conflicts of interest.

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