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Disseminated Mycobacteroides chelonae infection in a free-ranging jararaca (Bothrops jararaca)

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Abstract

Nontuberculous mycobacteria (NTM) are widely distributed in the environment and can cause infections in several animal species, including reptiles. However, reports of NTM infections in free-ranging reptiles remain scarce. This study describes the pathological and molecular findings of a disseminated *Mycobacteroides chelonae* infection in a free-ranging jararaca (*Bothrops jararaca*) from São Paulo, Brazil. An adult female snake found dead in the wild was submitted for post-mortem examination. Necropsy revealed multifocal to miliary yellowish granulomas in the liver and multifocal pulmonary nodules, as well as traumatic lesions. The snake was also gravid, with 32 fetuses at an advanced stage of development. Histopathological examination identified multifocal granulomatous hepatitis, granulomatous pneumonia with central necrosis, and granulomatous lesions in the splenopancreas. Ziehl–Neelsen staining revealed numerous acid-fast bacilli within hepatic and pulmonary granulomas. Molecular analysis of an 86-bp fragment of the mycobacterial *hsp65* gene showed 98.85% nucleotide identity with *M. chelonae* sequences available in GenBank. These findings are consistent with a disseminated mycobacterial infection attributed to *M. chelonae*. This case expands the known occurrence of NTM infections in reptiles and highlights the value of combining necropsy, histopathology, special stains, and molecular methods in the investigation of infectious diseases in wildlife. Further studies involving additional cases and more extensive molecular characterization may help clarify the occurrence and pathological features of NTM infections in free-ranging reptiles.

Background

Snakes of the genus *Bothrops* are an important public health concern in Brazil and are responsible for most reported snakebite envenomations (Cardoso et al. 2026). Among the species in this genus, *Bothrops jararaca* (Wied-Neuwied, 1824), a member of the family Viperidae, stands out for its wide distribution in southeastern South America, occurring from southern Bahia to southernmost Brazil, with records also in eastern Paraguay and Misiones Province, Argentina (Cacciali et al. 2019) (Fig. 1). This species exhibits remarkable ecological plasticity, inhabiting environments ranging from well-preserved Atlantic Forest areas to human-modified landscapes (Siqueira and Marques 2018).

Given the public health importance of these snakes, studies have used captive animals for venom extraction for snakebite antivenom production (Grego et al. 2021), and others have investigated bioactive venom compounds with pharmacological potential. Research on bradykinin-potentiating peptides isolated from *B. jararaca* venom contributed to the development of captopril, an angiotensin-converting enzyme inhibitor widely used to treat hypertension (Camargo et al. 2012; Sciani and Pimenta 2017). Studies of free-ranging *B. jararaca* include investigations of intestinal microbiota (Bastos et al. 2008), venom comparisons (Farias et al. 2018), and parasitic infections (Grego et al. 2004). Others have compared the protein composition and functional properties of venom from *B. jararaca* recently captured in the wild with that of individuals kept in captivity for extended periods (Galizio et al. 2018).

However, information on bacterial infections in this species remains limited. Among clinically relevant agents, mycobacteria stand out as aerobic, acid-fast bacteria that include members of the *Mycobacterium tuberculosis* complex (MTBC), which are associated with tuberculosis, and nontuberculous mycobacteria (NTM), which comprise other species capable of causing infections in humans and animals (Lopes et al. 2022; Pavlik et al. 2022). In snakes and other reptiles, mycobacterial infections are frequently associated with granulomatous lesions, which may be localized or disseminated (Soldati et al. 2004). Among NTM, *Mycobacteroides chelonae*, a member of the *M. chelonae–abscessus* complex (MCABC), is of particular interest because of its clinical relevance, its ability to cause infections in humans and animals, and its resistance to several classes of antimicrobial agents (Chong et al. 2022; Johansen et al. 2020; Medjahed et al. 2010).

In this context, this report describes a case of disseminated infection by *M. chelonae* in a free-ranging *B. jararaca* to characterize the pathological and molecular findings associated with the infection.

Case presentation

On January 24, 2023, an adult female jararaca (*Bothrops jararaca*), found outdoors in northern São Paulo city, Brazil, was received dead by the Wildlife Division (*Divisão da Fauna Silvestre*), a municipal wildlife screening center. The animal was classified as decomposition code 2 and refrigerated pending investigation of the cause of death.

At necropsy, the animal had a moderate blunt-force lesion over a lumbar vertebra (Fig. 2A) and a small, circular penetrating lesion measuring 0.6 × 0.5 cm in the right intercostal musculature, adjacent to the vertebral column (Fig. 2B).

The main gross findings were hepatic granulomas (Fig. 3A), pulmonary granulomas (Fig. 3B), and 32 fetuses at an advanced stage of development (Fig. 4A and B).

Histopathological examination was performed at the Pathology Center of the Adolfo Lutz Institute in São Paulo, Brazil. Tissue samples were fixed in 10% formalin, embedded in paraffin, cut into 4 µm sections, and stained with hematoxylin and eosin (H&E). Histopathology revealed multifocal granulomatous hepatitis with histiocytic granulomas, some of them focally extensive. The granulomas were composed of aggregates of macrophages surrounded by lymphocytic infiltrates and connective tissue and were associated with moderate hepatic steatosis (Fig. 5A). The lungs showed multifocal granulomatous pneumonia, with granulomas containing central necrosis (Fig. 5B). The splenopancreas exhibited moderate histiocytosis accompanied by multifocal granulomas. The kidneys showed moderate interstitial edema. No significant histological changes were observed in the intestine.

Ziehl–Neelsen staining was positive for acid-fast bacilli (AFB) in granulomas of the lung and liver (Fig. 6A and B), indicating a multibacillary process.

DNA was extracted from five 10 µm sections of formalin-fixed, paraffin-embedded (FFPE) liver and lung samples using the ReliaPrep™ FFPE gDNA Miniprep System (Promega, Madison, WI, USA), according to the manufacturer's instructions. A semi-nested polymerase chain reaction (PCR) targeting the 65-kDa antigen gene of mycobacteria was performed using specific oligonucleotide primers and GoTaq® Green Master Mix (Promega). PCR amplicons were purified using ExoSAP-IT™ (USB/Affymetrix, Santa Clara, CA, USA) and subjected to Sanger sequencing. Sequencing reactions were purified using the BigDye XTerminator™ Purification Kit (Applied Biosystems, Foster City, CA, USA) and analyzed on an Applied Biosystems 3500 Genetic Analyzer. The obtained sequences were assembled and manually edited using BioEdit version 7.2.5, and the resulting consensus sequence was compared with sequences available in the GenBank database using the Basic Local Alignment Search Tool (BLAST). BLAST analysis of the 86-bp consensus sequence showed 100% query coverage and 98.85% nucleotide identity with *Mycobacteroides chelonae*, with 198 database hits corresponding to this species. Among the closest matches were *M. chelonae* sequences deposited under GenBank accession numbers OQ571399, OQ571392, and OQ571389, obtained from isolates recovered from *Corydoras paleatus* and *Carassius auratus* in Turkey. The consensus sequence generated in this study was not deposited in GenBank because its length (86 bp) was below the minimum sequence length required for submission.

Discussion and conclusion

This report describes a disseminated infection attributed to *Mycobacteroides chelonae* in a free-ranging *Bothrops jararaca*. Reports of mycobacterial infections in reptiles remain limited (Ebani et al. 2012; Ullmann et al. 2016; Goldberg et al. 2023).

In Brazil, bovine and buffalo tuberculosis are addressed by the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT), which provides measures for disease diagnosis, surveillance, and control. *Mycobacterium bovis* is a major etiological agent of tuberculosis in these species (Carneiro and Kaneene 2018; Dametto et al. 2020; Bezerra Neto et al. 2024).

In contrast, knowledge regarding the occurrence and distribution of NTM in animals remains more limited, particularly in wildlife, for which available records frequently originate from isolated studies or animals maintained in captivity (Portas et al. 2009; Schrenzel et al. 2008; Lécu and Ball 2011; Vetere et al. 2022; Zeineldin et al. 2023).

The MCABC comprises rapidly growing nontuberculous mycobacteria that have been described as causative agents of infections in different hosts and clinical settings. These bacteria exhibit resistance to several classes of antimicrobial agents, which may complicate the treatment of the infections they cause (Victoria et al. 2021; Simões et al. 2023). In addition, species belonging to this complex are widely distributed in the environment and have been detected in various aquatic and terrestrial habitats, potentially exposing animals through contact with contaminated environmental sources (Parsons et al. 2022; Gebert et al. 2026; Fogelson et al. 2019).

Infections caused by mycobacteria belonging to the MCABC have been described in reptiles, including captive snakes (Simões et al. 2023) and animals from zoological institutions (Ebani et al. 2012; Soldati et al. 2004; Ullmann et al. 2016). In snakes, clinical and pathological manifestations may include localized or disseminated granulomatous lesions, although transmission mechanisms and factors associated with the establishment of infection remain incompletely understood (Vetere et al. 2022). In free-ranging reptiles, however, reports are less frequent, particularly regarding disseminated NTM infections (Donnelly et al. 2016; Goldberg et al. 2023).

Environmental exposure is a potential route of mycobacterial infection in reptiles and may occur through inoculation via skin lesions or ingestion of microorganisms present in the environment. The wide environmental distribution of *M. chelonae*, including its presence in aquatic and terrestrial sources, represents a potential source of exposure for free-ranging animals (Glauser et al. 2024; Glickman et al. 2020). In the present case, the circular penetrating lesion in the intercostal musculature raises the possibility of traumatic inoculation, but a causal relationship between this lesion and the infection could not be established. The animal was carrying 32 fetuses at an advanced stage of development; however, the relevance of this finding to the infection is unknown.

The lesions most frequently associated with mycobacteriosis in reptiles are granulomatous and may affect different organs and tissues, reflecting, in many cases, the chronic nature of the infection (Soldati et al. 2004; Mitchell 2012). In the present case, the presence of granulomatous lesions in multiple organs, together with the detection of acid-fast bacilli and the molecular identification of *M. chelonae*, is consistent with a chronic disseminated infection.

In this case, necropsy, histopathological examination, special stains, and molecular methods were used together to investigate lesions suggestive of mycobacteriosis. The detection of acid-fast bacilli by Ziehl–Neelsen staining supports a presumptive diagnosis of mycobacterial infection but does not allow identification of the causative agent at the species level. Molecular techniques, such as PCR and sequencing, are required for this purpose, particularly given the diversity of NTM and their similar phenotypic characteristics.

Systematic investigations of mycobacterial infections in free-ranging reptiles may help characterize the occurrence and distribution of these agents in wild populations. Furthermore, wildlife rehabilitation and screening centers may represent sources of information for infectious disease surveillance, particularly because they receive animals from diverse environmental settings and areas with varying degrees of interaction with urban and peri-urban environments.

Human infections caused by members of the MCABC have been described both in individuals with risk factors and in patients without recognized predisposing factors, and may present as localized or disseminated disease (Johansen et al. 2020). Although the epidemiological relationships among infections observed in humans, animals, and the environment remain incompletely understood, the occurrence of these bacteria in wildlife raises questions regarding their environmental persistence and potential relevance at the human–animal–environment interface.

The identification of *Mycobacteroides chelonae* in a free-ranging *Bothrops jararaca* adds to the documented occurrences of nontuberculous mycobacterial (NTM) infections in reptiles and provides a pathological description of disseminated infection in this snake species under natural conditions. The presence of granulomatous lesions in multiple organs, together with the detection of acid-fast bacilli and molecular findings consistent with *M. chelonae*, supports the use of an integrated diagnostic approach when investigating suspected mycobacterial infections in wildlife.

This report is limited to a single animal and does not allow conclusions regarding the frequency, origin, transmission, or population-level impact of NTM infections in free-ranging reptiles. Further investigations should include systematic assessment of granulomatous lesions in wildlife, combining histopathology, special stains, microbiological culture, and more extensive molecular characterization whenever feasible. The accumulation of comparable case data may help clarify the range of affected species, the pathological patterns associated with NTM infections, and their occurrence across different environments.

Declarations

Author Contribution

Author Contributions: Paulo Vouvouloudas contributed to the conceptualization and writing of the case report, data curation, and integration of the clinical, pathological, and laboratory findings. Ticiana Zwarg, Thaís C. Sanches, and Mayra H. Frediani performed the necropsy, including organ collection for histopathological and molecular analyses, and critically revised the manuscript. Juliana Mariotti Guerra developed and applied the methodology for histopathological analyses and molecular detection of the agent, and critically revised the manuscript. Eduardo Ferreira Machado performed the microscopic examination of the histological slides and prepared the photomicrographs. Leonardo José Tadeu de Araújo and Natalia C. C. A. Fernandes performed the histopathological and molecular analyses. All authors have read and approved the final version of the manuscript.

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Figures

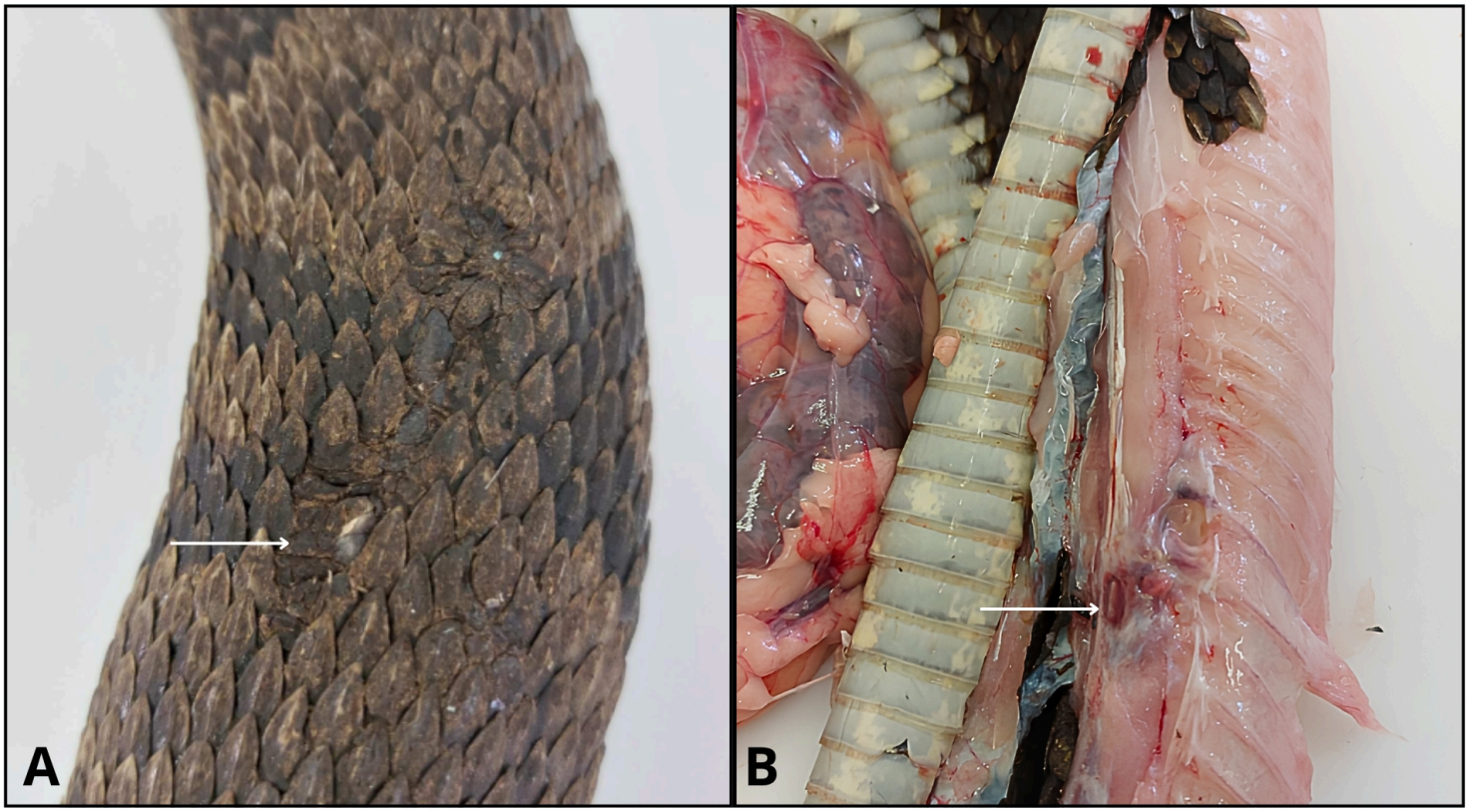


Figure 1

Geographical distribution of *Bothrops jararaca*. Left: illustration of the species; right: known geographical distribution, which includes southern Bahia and southern and southeastern Brazil and extends into eastern Paraguay and Misiones Province, Argentina. Adapted from The IUCN Red List of Threatened Species.

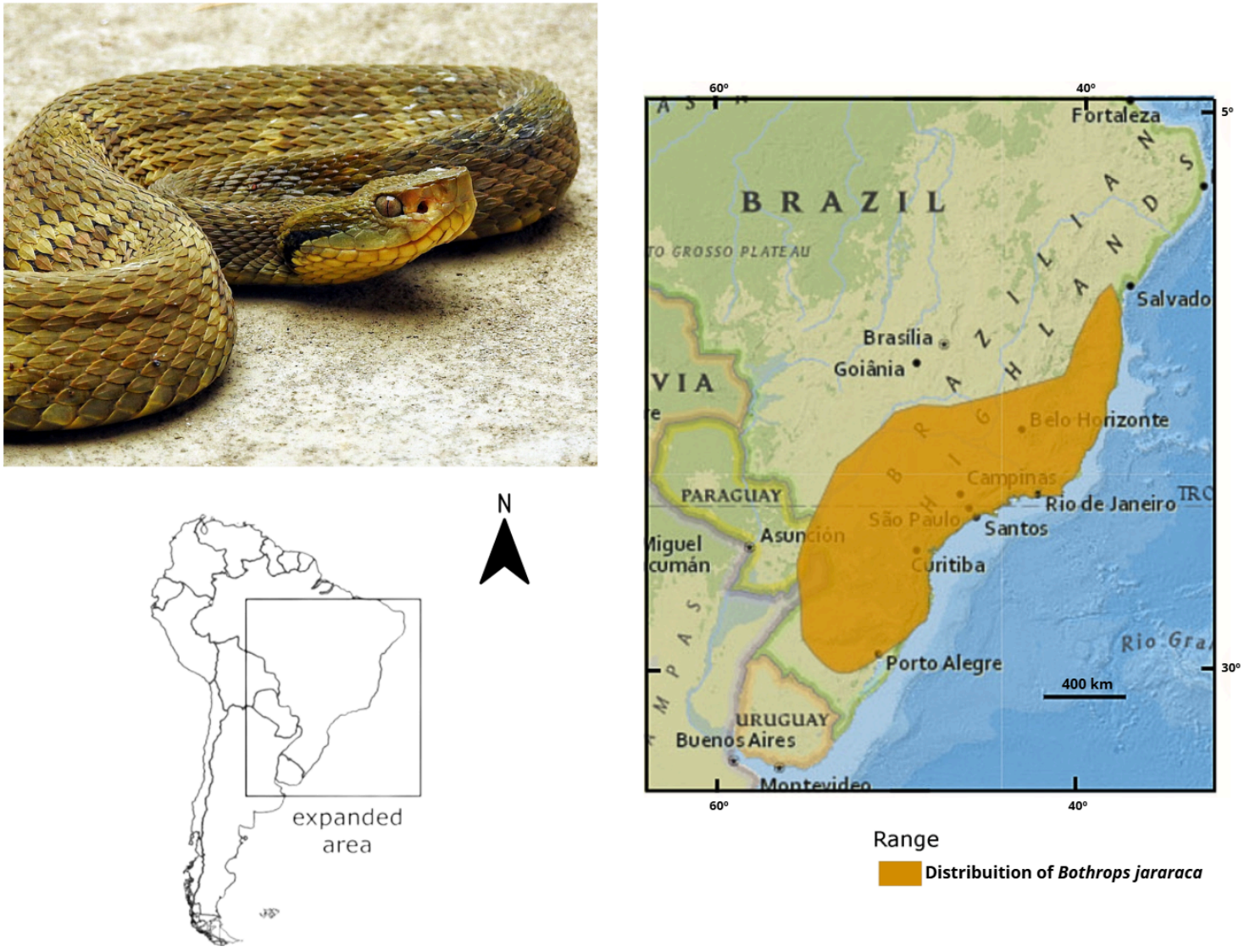


Figure 2

Blunt-force and penetrating lesions identified during necropsy. **A:** Blunt-force lesion (white arrow) over a lumbar vertebra. **B:**Circular penetrating lesion (white arrow) in the right intercostal musculature. Source: Wildlife Division, 2023.

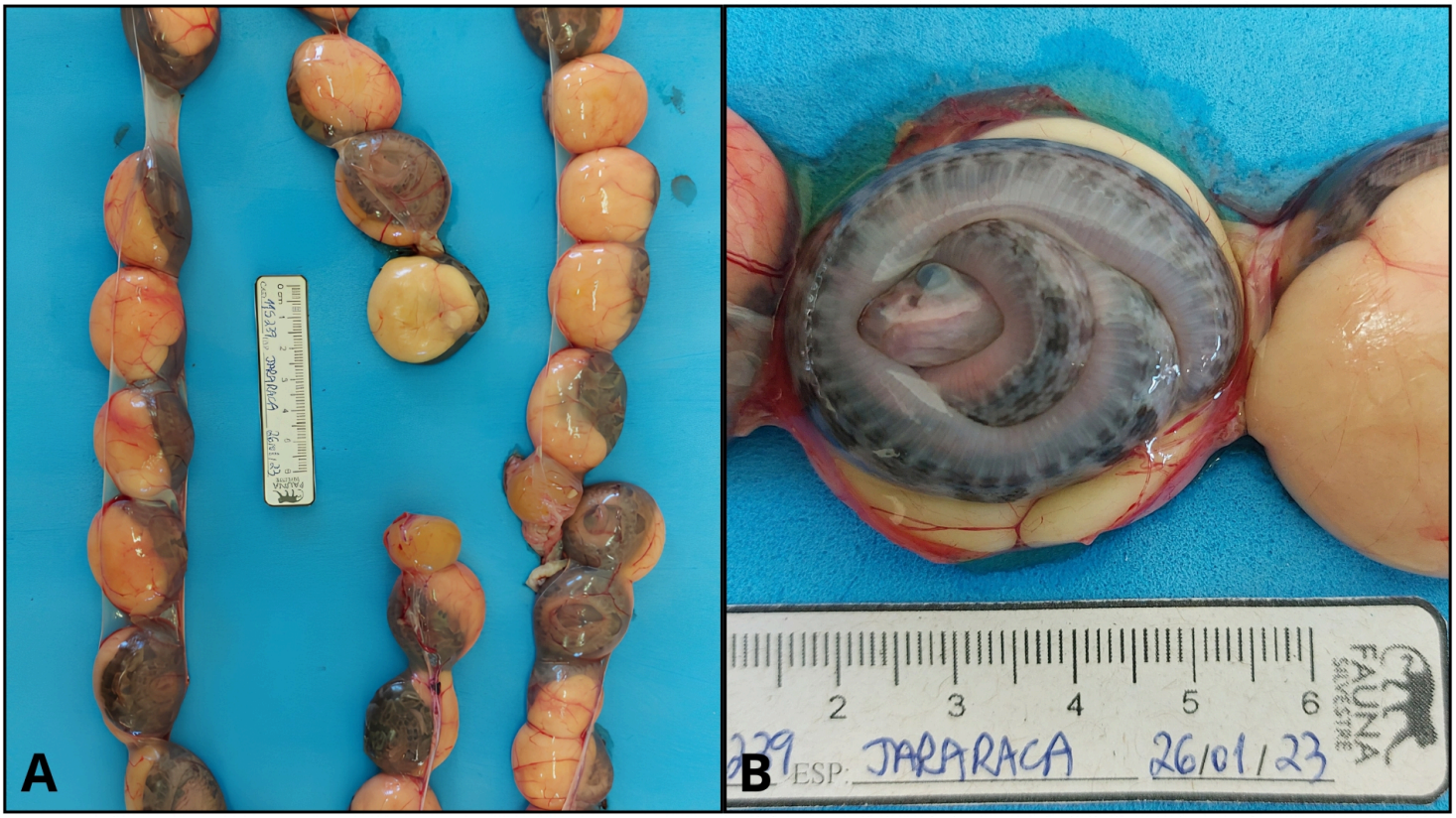


Figure 3

Granulomas identified during organ examination. **A:**Liver with small, yellowish, pinpoint lesions (white arrow), measuring less than 0.1 cm, with a multifocal to miliary distribution that remained visible on the cut surface. **B:** Lung with small, yellowish nodules (white arrow), approximately 0.1 cm in diameter, with a multifocal distribution. Source: Wildlife Division, 2023.

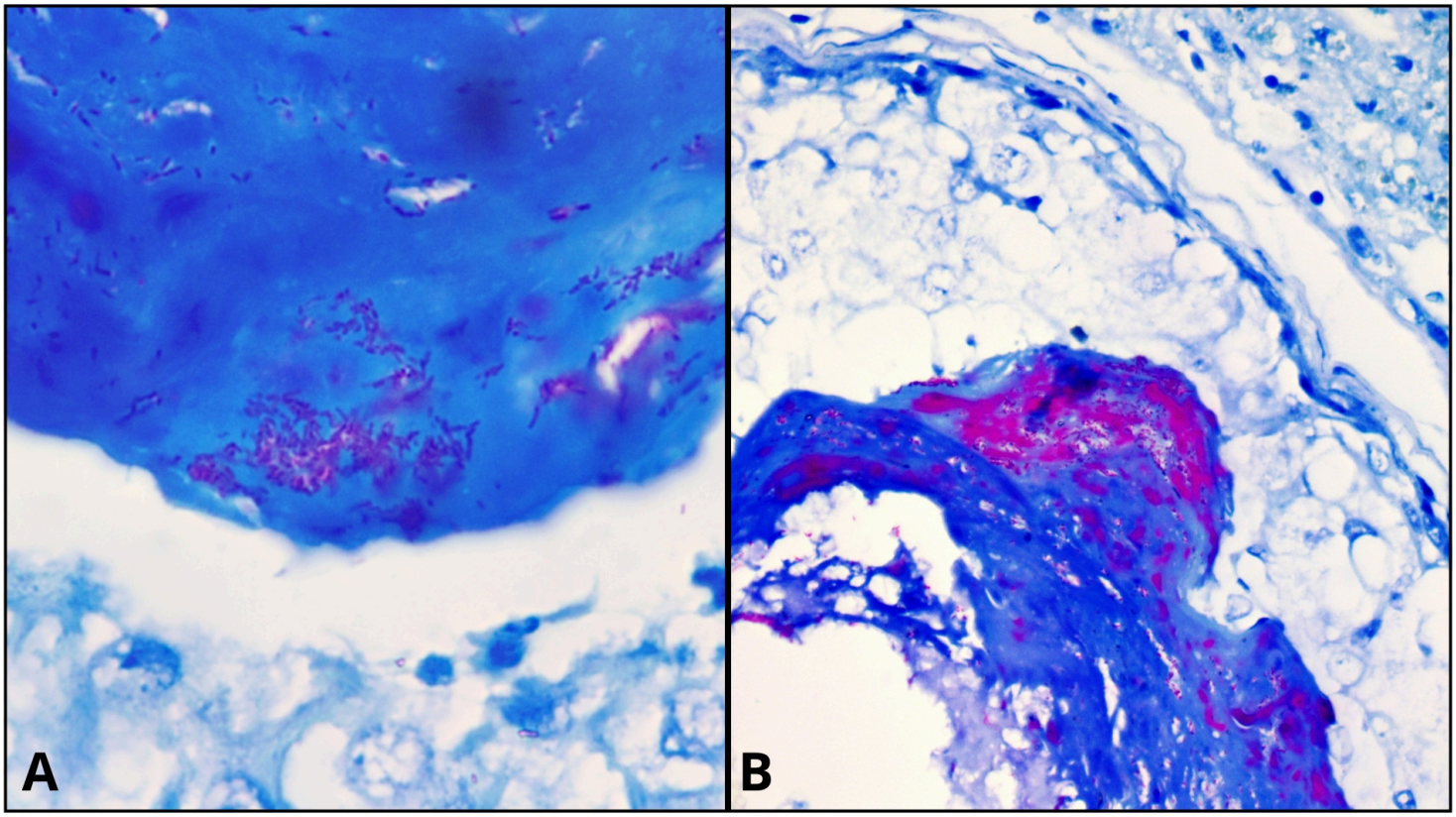


Figure 4

Evidence of the animal's pregnancy status. **A:** The animal carried 32 fetuses at an advanced stage of development. **B:** Sectioned fetus showing advanced developmental features . Source: Wildlife Division, 2023.

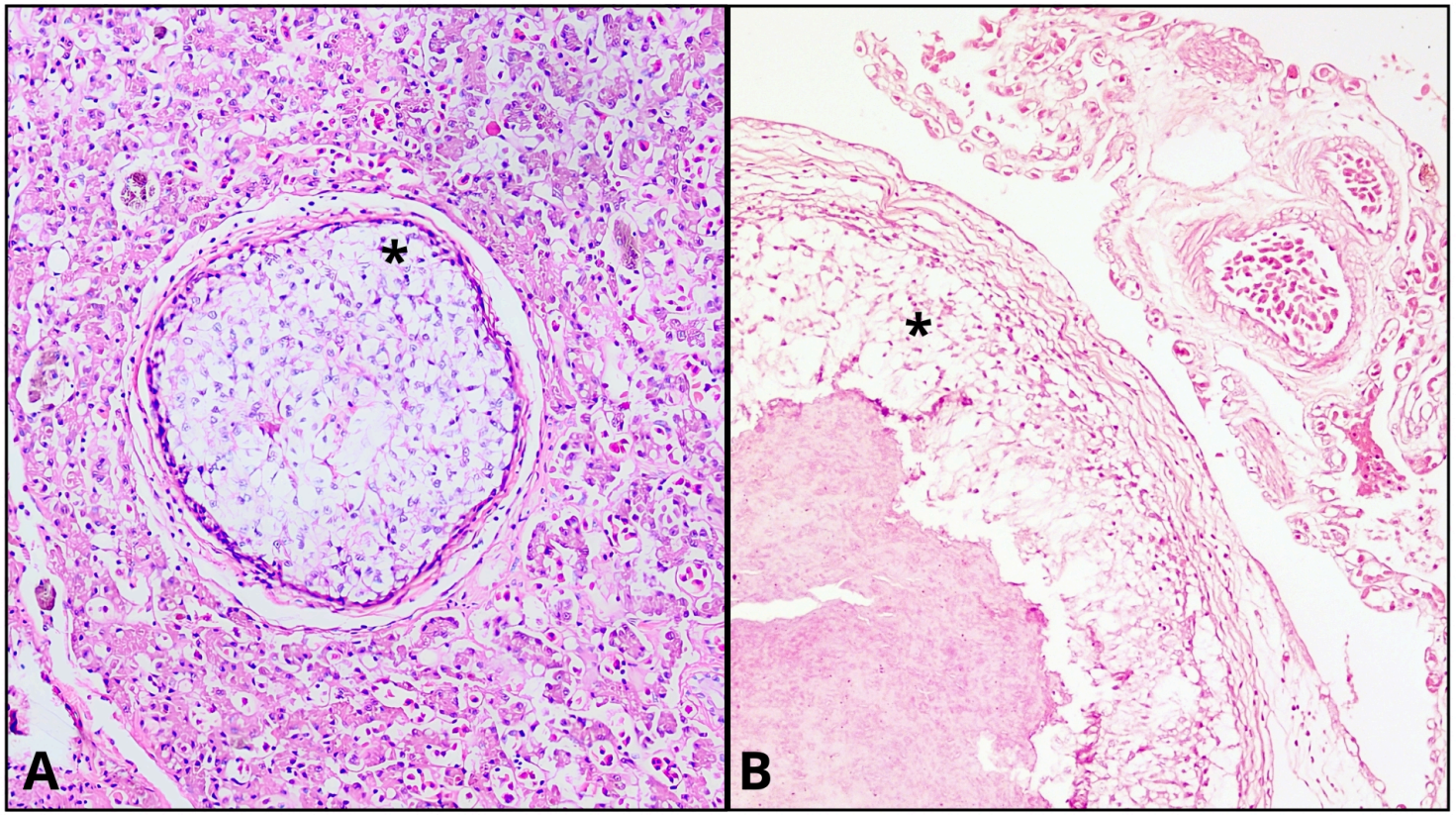


Figure 5

Histopathology of the liver and lung of a jararaca (*Bothrops jararaca*) infected with *Mycobacteroides chelonae*. **A:** Liver showing a focally extensive histiocytic granuloma and atrophy of adjacent hepatocytes. H&E, 10× objective. **B:** Lung showing a histiocytic granuloma (*) with central necrosis, surrounded by a capsule. H&E, 100× objective. Source: Adolfo Lutz Institute.

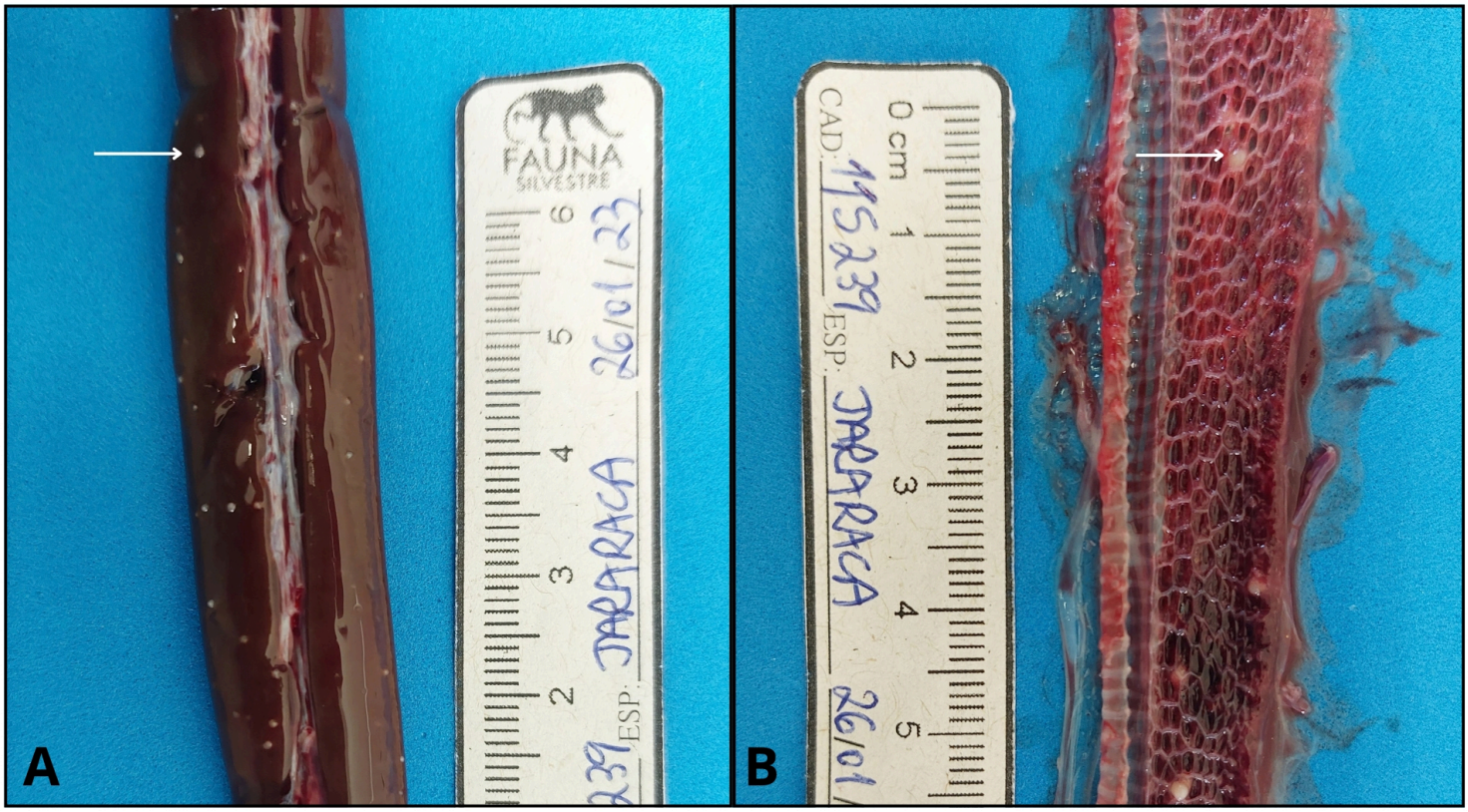


Figure 6

Ziehl–Neelsen staining of the liver and lung of a jararaca (*Bothrops jararaca*) infected with *Mycobacteroides chelonae*. Acid-fast bacilli (AFB) within granulomas, indicating a multibacillary process. **A:** Lung, Ziehl–Neelsen, 100× objective. AFB in the center of a granuloma. **B:** Liver, Ziehl–Neelsen, 400× objective. Numerous AFB in the center of a granuloma. Source: Adolfo Lutz Institute.