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Pathological and incidental findings in anurans from Ceará, northeastern Brazil

Achados patológicos e incidentais em anuros do Ceará, nordeste brasileiro

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Abstract: Amphibians are among the most threatened animal groups on Earth, with anurans representing the most prevalent order within this group. Globally, emerging infectious diseases are linked to the decline of amphibian populations, a phenomenon also observed in northeastern Brazil. In particular, the state of Ceará boasts a rich and abundant anurofauna, with nearly 5% of its species considered critically endangered. Despite numerous pathologies observed in local anurans through research projects, published cases remain scarce. This study aimed to compile pathological and incidental findings in native anurans from Ceará State, Northeastern Brazil. Data were derived from necropsies and clinical examinations conducted on 38 specimens across 13 species from 13 sites, spanning from 2010 to 2022. Most lesions (71%, n = 38) indicated inflammatory pathophysiology, with observations of parasitic infections and agents within lesions, granulomatous or necrotic lesions with intracytoplasmic inclusions consistent with *Mycobacteria* and Ranavirus infections, respectively. Fibrolipomas and hepatocellular carcinoma presented as single solid nodules, the latter associated with cystic helminth infections. Diffuse hepatic calcinosis suggested a toxic/metabolic etiology. Bilateral cataract was the most common ocular alteration (60%, n=5), though its etiology remained undetermined. The presence of infectious diseases was confirmed, and further etiological diagnostics are necessary. The lack of specific etiological techniques constrained some definitive diagnoses. These findings place Ceará on the distribution map for significant diseases affecting anurofauna, underscoring the need for ongoing monitoring.

Keywords: amphibians; cataract; infectious diseases; necropsy; neoplasms.

Resumo: Anfíbios são um dos grupos de vertebrados mais ameaçados globalmente, e os anuros são sua ordem mais representativa. Doenças infecciosas emergentes têm sido associadas ao declínio global das espécies de anfíbios, fenômeno relatado em todo o mundo. Na região Nordeste, o Ceará tem uma abundante anurofauna, com cerca de 5% de suas espécies sendo consideradas criticamente ameaçadas. Em projetos de pesquisa, várias patologias são observadas em anuros

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silvestres locais, contudo, poucos casos têm sido publicados. O objetivo deste trabalho foi reunir achados patológicos e incidentais em anuros nativos do estado do Ceará, nordeste do Brasil. Os achados foram registrados durante necrópsias e exames clínicos. A amostra incluiu 38 espécimes, distribuídos em 13 espécies, originários de 13 localidades, examinados entre 2010 e 2022. A maioria das lesões (71%, n = 38) apontou para fisiopatologia inflamatória, incluindo infecções parasitárias com agentes lesionais - lesões granulomatosas e necrotizantes com inclusões intracitoplasmáticas, compatíveis com *Mycobacteria* e Ranavírus, respectivamente. Fibrolipoma e carcinoma hepatocelular se apresentaram como nódulos únicos, estando o último associado a uma infecção helmíntica cística. Calcinose hepática difusa representou doença de etiologia tóxico/metabólica. Catarata bilateral foi a alteração ocular mais frequente (60%, n=5), mas sem diagnóstico etiológico conclusivo. Indícios de doenças infecciosas foram detectados e necessitam de técnicas complementares de diagnóstico etiológico. A falta de laboratórios locais ou parceiros com técnicas diagnósticas específicas limitou alguns diagnósticos definitivos. Os achados aqui apresentados colocam o estado do Ceará no mapa de doenças preocupantes da anurofauna, que necessitam ser monitoradas.

Palavras-chave: anfíbios; catarata; doenças infecciosas; necrópsia; neoplasmas.

1. Introduction

The Order Anura, encompassing approximately 7,568 species[1], represents the most diverse group among amphibians and is distributed across virtually all continents, excluding frozen regions[2]. These populations are highly susceptible to environmental variations[3] due to their unique metabolic and reproductive physiology. Anurans exhibit diverse reproductive modes, each reliant on specific and optimal environmental conditions[4,5]. Consequently, they are among the most endangered animal groups globally[6].

Critical diseases impacting amphibians include infections from *Batrachochytrium dendrobatidis*, commonly known as 'chytrid,' and Ranavirus, both of which are linked to the global decline of these species[7,8,9]. Chytrid infections have been documented in several Brazilian species for at least 15 years[10,11], while Ranavirus has only recently been detected in wild populations[12]. These infectious and parasitic diseases can severely affect amphibian populations and communities, with their impact potentially exacerbated by climate change, habitat loss, pollution, and inbreeding depression in small populations[13]. Moreover, *Mycobacterium* sp. and fungal infections may affect amphibians and concern public health[14,15,16].

In the state of Ceará, 57 amphibian species have been recorded[17,18], including four endemic species, three of which are considered critically endangered[19]. The presence of Ranavirus and chytrid heightens concerns for these endemic and endangered species[20]. Although these diseases have not been formally reported in Ceará, they have been identified in neighboring states[21,22]. Moreover, less prominent pathologies have been observed in local native frogs and toads but remain largely unpublished or only briefly mentioned in ecological studies. For instance, Mycobacteria have occasionally been reported (Braga, unpublished data) and were recently detected by molecular diagnostics in local anurans[23]. Additionally, ecto and endoparasites, often discussed in taxonomic and ecological studies[24,25,26,27], seldom have their pathogenic impacts examined. Some metabolic changes, such as organic mineralizations, typically seen in livestock(28), are rarely reported in wild species[28] and only once in amphibians (Braga, unpublished data).

This study aimed to compile pathological and incidental findings in native anurans from Ceará, northeastern Brazil, assessed over more than a decade.

2. Material and methods

2.1. Study areas, sampling and ethics

From 2010 to 2022, various species of anurans were collected across different municipalities in Ceará State, Northeastern Brazil, as part of postgraduate research projects and local fauna monitoring efforts. The collection of these individuals was authorized under multiple permits (DCL #22909-1; DPC # 39073-4; PMG # 58724-1; RWA #29613, RRB #82318) issued by the Biodiversity Authorization and Information System (SisBIO) for the duration of each research project. The sampling approach was opportunistic.

The methods for collecting these species included active searching or using pitfall traps during crepuscular and nocturnal times. Upon capture, the individuals were identified and measured for snout-vent length (SVL) and body mass. Depending on the specific aims of each project, some anurans were examined and then released immediately after having blood drawn for clinical pathology tests and receiving a subcutaneous microchip. Others were euthanized through intracranial injection of an overdose of lidocaine hydrochloride, as per the protocols outlined by Chatigny et al.[30], adhering to the standards set by the Federal Council of Veterinary Medicine[31], and subsequently the guidelines of the National Council for the Control of Animal Experimentation (CONCEA)[32]. Some of these activities were also authorized by CEUA-USP # 4387250118.

2.2. Necropsy and laboratory techniques

Laboratory diagnostics included hematological analysis from blood smears and cytopathology from touch impressions or aspirations from external lesions, following the methods described by Campbell[33]. Pathological or incidental findings were derived from systematic necropsies, as outlined by Pessier and Pinkerton[34]; clinical examinations; and dissections for collecting tissues, diet, and parasites. The organs and tissues showing alterations were macroscopically described and samples were taken for histopathological evaluation, following the procedures recommended by Tadrous[35]. Granulomatous lesions were specifically stained using Ziehl-Neelsen (ZN) and Wade-Fite (WF) techniques, as per the protocols of Azulay & Andrade[36] and Greenwood & Fox[37]. Subsequently, the sampled individuals were fixed in 10% formaldehyde, preserved in 70% ethanol, and deposited in the Herpetological Collection of the Federal University of Ceará (CHUFC).

2.3. Data analysis

The observed alterations in the anurans were categorized into four primary types: infectious-parasitic/inflammatory, toxic-metabolic, traumatic, and neoplastic. Data were organized into contingency tables featuring individual identification, species, origin (Capital city, Metropolitan Area, and Countryside), sample size, and the categories of alterations along with their respective frequencies. Prevalence, classifications, and sites of occurrence were expressed as percentages. A proportions test was conducted to compare the main prevalence from different origins, hypothesizing that less anthropized locations would exhibit lower prevalence. Statistical analyses and the creation of maps were performed using the R program version 4.2.2[38].

3. Results

3.1. Included specimens and geographical origin

From 2010 to 2022, 44 anuran specimens across 13 species (Table 1) were examined, originating from 13 different sites (Figure 1). Six of these specimens were excluded from the final analysis as they had previously been published in conference proceedings. Therefore, the final sample size consisted of 38 specimens.

Table 1. Pathological and Incidental Findings in Anuran from Ceará. Species and sample size by locality

SPECIES	LOCALITY (QUANTITY)	SUBTOTAL
Adelophryne maranguapensis	Maranguape (n=4)	4
Boana raniceps	Maranguape (n=1)	1
Leptodactylus fuscus	Fortaleza (n=1)	1
Leptodactylus macrosternum	Aiuaba (n=5), Fortaleza (n=2), Itarema (n=1), Limoeiro do Norte (n=2), Pacatuba (n=1), Tabuleiro do Norte (n=3)	14
Leptodactylus troglodytes	Pacatuba (n=1)	1
Leptodactylus vastus	Fortaleza (n=2), Maranguape (n=1), Pacatuba (n=1), Tianguá (n=2)	6
Physalaemus cuvieri	Pentecoste (n=1), Ubajara (n=1)	2
Pleurodema diplolister	Maranguape (n=1)	1
Pristimantis relictus	Maranguape (n=1)	1
Proceratophrys cristiceps	Crateús (n=1), Itapipoca (n=1), Pecém (n=1)	3
Rhinella diptycha	Fortaleza (n=2), Ubajara (n=1)	3
Trachycephalus typhonius	Maranguape (n=1)	1
TOTAL		**38**

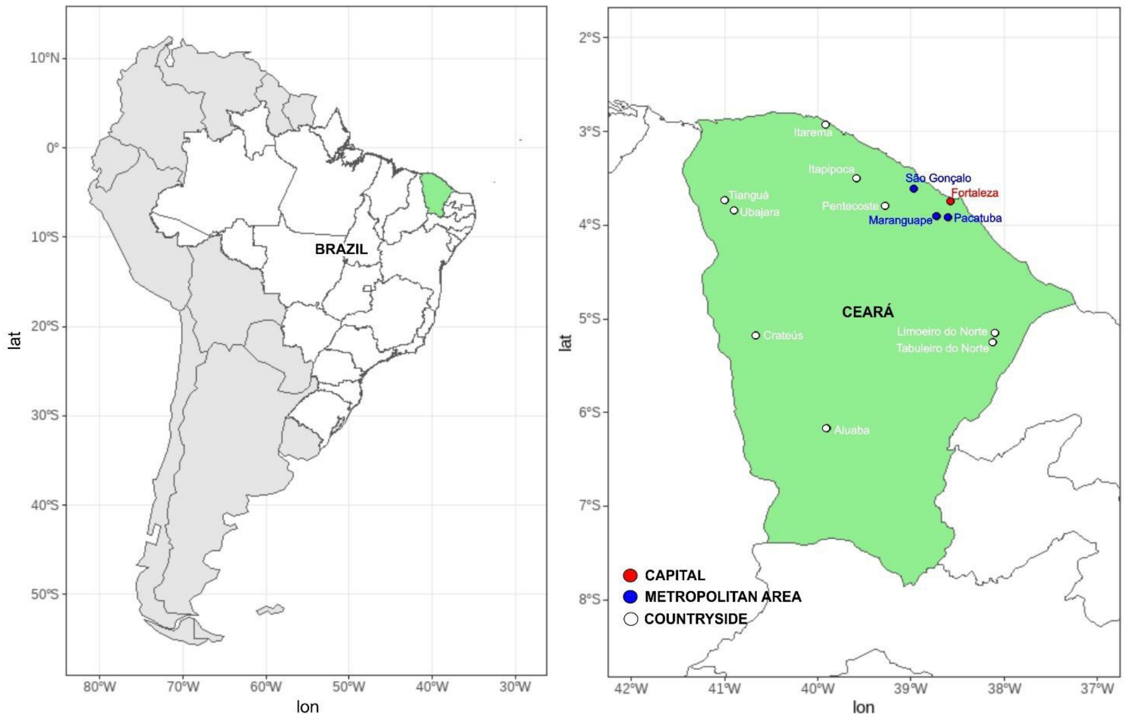

Figure 1. Pathological and Incidental Findings in Anuran from Ceará. Sample geographical origin.

According to geographical origin, the sample included seven individuals from Fortaleza, 13 from the metropolitan area, and 18 from the countryside.

3.2. Pathological findings and etiologies

Displays the pathological findings categorized by presumable etiology and lesion sites.

Table 2. Pathological and Incidental Findings in Anurans from Ceará State, Brazil. Observed Lesions, Counting, and Distribution.

					Lesion site					
Etiological classification	Spleen	Cavity	Stomach	Liver	Skeletal-muscle	Eyes	Kidneys	Systemic	Integument	Total
Infectious/Inflammatory	3	2	1	2	2	1	2	11	3	**27**
Benign Neoplasm									1	**1**
Malign Neoplasm				1						1
Toxic/Metabolic				1						1
Traumatic					3					3
Non classified						5				5
Total	**3**	**2**	**1**	**4**	**5**	**6**	**2**	**11**	**4**	**38**

Approximately 60% (22/38) of diagnoses were made from necropsies followed by histopathological evaluation. Other methods included necropsy alone (10/38), clinical follow-up with subsequent necropsy and histopathology (2/38), clinical pathology only (2/38), and clinical observation only (2/38). Among the histopathological evaluations, 15 out of 22 cases involved the examination of all coelomic organs, whereas in the remaining cases, only the affected organ was examined. Over 70% (27/38) of the cases involved single findings, while 11 out of 38 cases had multiple findings (ranging from two to four) in different organs. Approximately 45.4% (5/11) of these multiple findings could be traced back to the same toxic or infectious-parasitic etiology.

In terms of the lesions observed, 71% (27/38) indicated an inflammatory pathophysiology, likely of infectious-parasitic origin. Of these, 40.7% (11/27) were classified as systemic alterations, affecting multiple organs simultaneously or involving blood alterations. While the primary etiology of observed alterations could not always be confirmed, specific findings included 10/27 cases of parasitic infections with identifiable intralesional agents, 5/27 potential bacterial causes, 5/27 potential viral causes, and 5/27 cases remained inconclusive. Evidence of multiple etiologies was detected in 4/27 cases. These findings are detailed in Figure 2.

In parasitic infections (Figure 2A-E), a range of manifestations were observed: mild multifocal parasitic dermatitis due to Trombiculidae (1/10); mild subacute multifocal granulocytic parasitic pneumonia with intralesional Nematoda (*Rhabdias* sp.) (1/10); moderate multifocal cystic hepatitis with intralesional Acanthocephala (1/10); mild chronic multifocal granulomatous splenitis caused by Haemogregarinidae meronts (2/10); chronic moderate multifocal cystic subserous gastritis from Acanthocephala larvae (*Centrorhynchus* sp.) (1/10); and focal pyogranulomatous or hemorrhagic cystic mural/cavitary myositis with an intralesional nematode (2/10), specifically in the left submandibular (1/10) and left ventral femoral (1/10) regions. Additionally, a neoplasm with intralesional Nematoda was observed, which will be elaborated upon later. One individual exhibited moderate chronic multifocal interstitial cystic parasitic nephritis with intralesional Nematoda and specific granulomas, contributing to the bacterial etiologies discussed below.

Among the lesions with probable viral origins (5/27), one individual without detectable clinical signs showed eosinophilic intraerythrocytic inclusions on a blood smear, consistent with *Frog Erythrocytic Virus* (FEV, Iridoviridae, Figure 2F). The other four cases (4/5) featured amphophilic intracytoplasmic inclusions in the liver, kidney, and spleen tissues (Figure 2G), indicative of Ranavirus.

Among the probable bacterial etiologies, the most common findings were granulomatous hepatitis (3/5), splenitis (2/5), and nephritis (2/5). The granulomas were multifocal to coalescent, encapsulated, and multilocular, ranging from 30 to 550μm in diameter. They contained macrophages and melanoma-macrophages with phagocytosed particles. The granulomas showed inconclusive ZN and WF stains, with diffuse magenta staining within the granulomas, but without any stained bacilli or bacillary globi (Figure 2H). Additionally, one individual exhibited cutaneous nodules with pyogranulomatous inflammation upon cytological examination, revealing foamy macrophages with intracytoplasmic negatively staining bacillary particles, indicative of cutaneous mycobacteriosis. One of the 27 cases displayed fibrosing blepharitis accompanied by bilateral mature cataracts, described macroscopically. This condition was presumed to be a feature of an infectious disease, most likely of viral origin.

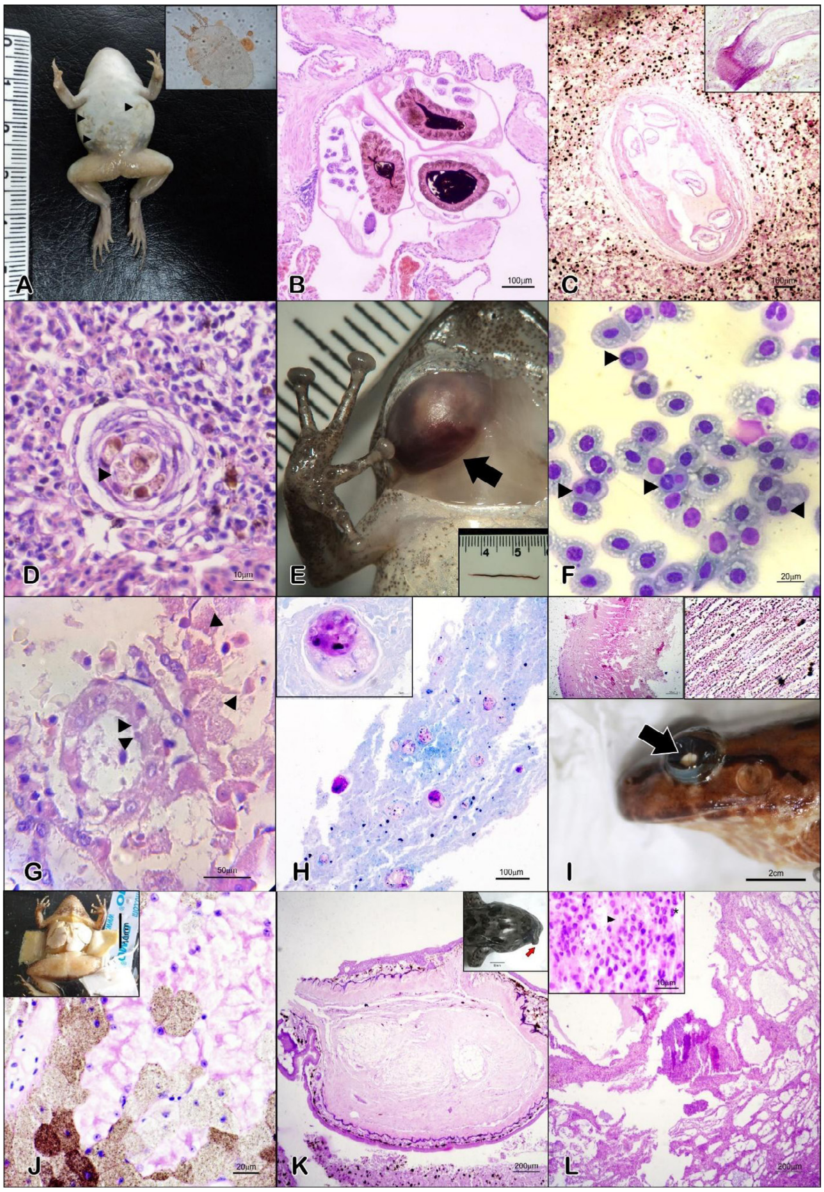

Figure 2. Macroscopic and Microscopic Pathological Findings in Anuran from Ceará. (A) *P. diplolister*, multifocal parasitic dermatitis (arrowheads) due to Trombiculidae mites (topright, 10% NaOH). (B) *R. diptycha*, parasitic pneumonia with intralesional Nematoda (*Rhabdias* sp., HE, 100x). (C) *L. vastus*, multifocal cystic hepatitis (HE, 100x), with intralesional Acanthocephala (top right, HE, 400x), and diffuse melanoma-macrophage proliferation. (D) *L. macrosternum*, multifocal granulomatous splenitis, by Haemogregarinidae meronts (arrowhead, HE, 1000x). (E) *P. relictus*, left submandibular focal hemorrhagic cystic myositis (arrow) with an intralesional nematode (bottom right). (F) *L. macrosternum*, eosinophilic intraerythrocytic inclusions, compatible with FEV (Panótico, 400x). (G) *L. macrosternum*, amphophilic intracytoplasmic inclusions (arrowheads) in degenerated kidney tubular cells, suggesting Ranavirus infection (HE, 400x). (H) *L. macrosternum*, multifocal granulomatous hepatitis, containing macrophages and melanoma-macrophages with phagocytosed particles (WF, 40x), with diffuse magenta-stained granuloma-like structures (top left, WF, 1000x). (I) *L. vastus*, bilateral mature cataract (arrow), moderately degenerated secondary lens fibers (top left, HE, 40x), suggestive diffuse Morgagnian globules in the primary lens fibers (top right, HE, 1000x). (J) *P. cuvieri*, diffuse mineralized and enlarged liver (top left), diffuse hepatic calcinosis/silicosis, showing diffuse degenerated hepatocytes and swollen-brown-particles-containing Kupffer cells (HE, 400x). (K) *L. fuscus*, right anteorbital subcutaneous nodule (top right) classified as a focal fibrolipoma (HE, 40x). (L) *B. raniceps*, hepatocellular carcinoma (HE, 40x), trabecular type along five to ten or more cells width, with at least two encysted *Physaloptera* sp. (not shown); pleomorphic hepatocytes, increased nucleus: cytoplasm rate, eventual vacuolation (arrowhead) and binucleated cells (asterisk), and irregular nuclear chromatin pattern distribution, from fine peripheral to coarse (HE, 1000x).

The overall prevalence of infectious-parasitic/inflammatory etiologies varied from 61.5% in the metropolitan region to 77.7% in the countryside; however, a proportions test revealed no significant differences among the prevalences from different origins (X-squared = 0.074044, df = 2, p-value = 0.9637). Nearly 8% (3/38) of the cases were classified as toxic-metabolic, including significant diffuse hepatic calcinosis/silicosis (Figure 2J). Another 8% (3/38) consisted of traumatic injuries, such as limb or digit amputations (2/3), hip dislocation (1/3), and skin erosions or ulcerations (1/3). Additionally, two dorsal fibrinonecrotic tegumentary lesions were documented; these were linked to internal alterations suggestive of a potential systemic clinical sign. About 5% (2/38) of the cases involved neoplasms: one focal fibrolipoma in the right anteorbital region, and a moderately differentiated hepatocellular carcinoma, each accompanied by at least two encysted *Physaloptera* sp. (Figure 2K-L). Finally, another 13% (5/38) of the findings were eye injuries grouped as "unclassified," which included bilateral cataracts (3/5, Figure 1I), unilateral exophthalmos (1/5), and presumed unilateral retinal detachment (1/5). These conditions may be related to aging, infectious, traumatic, neoplastic, or toxic-metabolic etiologies.

4. Discussion

Histopathological findings were documented in 13 anuran species from 13 sites in Ceará State, Brazil. These species, which are representative of the state's biodiversity(39), inhabit varied phyto-ecological zones such as the Caatinga (drylands), Brejos-de-Altitude (relictual Atlantic Forest highlands), and Matas de Tabuleiro (seasonally dry Atlantic Forest fragment), exposing them to distinct environmental pressures(40,41,42,43). The most frequently sampled species was *L. macrosternum* (n=8), which has a wide distribution across South America(44) and is found in diverse habitats, including highly anthropized areas(45). This species was recently evaluated as a bioindicator of chemical contamination by heavy metals in an agrosystems area in Ceará, indicating a significant probability of endocrine and lymphoid alterations(46,47).

The lesions observed stemmed from various pathological processes, suggesting specific etiologies. Challenges such as the absence of specialized microbiological laboratories limited the identification of viral and bacterial diseases. However, histopathological markers highlighted the need for ongoing monitoring and the development of research collaborations aimed at reaching definitive diagnoses.

Concerning parasitic findings, granulomatous dermatomyositis caused by Trombiculidae mites in an endangered native species in central Chile's coastal forest was reported with a high incidence rate (70%; 35/50), with encysted larvae present in the spongy layer of both ventral and dorsal dermis and muscle tissues of the hind limb, although the impact on host fitness was not determined(48). Helminths are commonly found in adult vertebrates' intestines; however, Cestoda larvae can induce hemorrhage and inflammation, while Nematoda may encyst in visceral cavities(49) or muscles(50), generally representing infective larval stages in accidental or paratenic hosts(51,52,53). Minimal inflammatory reactions to encysted Physalopteridae in stomachs have been observed(54). *Rhabdias* spp. respiratory infections might lead to secondary bacterial infections, suppurative pneumonia, and necrosis(55), adversely affecting performance, dispersion, foraging, and predation avoidance[56,57]. Meanwhile, the myositis described in this study involved hemorrhage and mild granulocytic infiltrate, compressive muscular hypotrophy, and cavity edema, with encysted Ascarididae larvae found in the coelomic wall. The pathology associated with Acanthocephala in ectothermic vertebrates is less understood, except for noted erosive damage to organs and tissues in other ectothermic or experimental species[58,59].

Anurans are infected by numerous parasites throughout their lifetimes[60], particularly during larval stages, likely due to their pivotal roles in ecosystemic food webs[61]. Parasites significantly impact individual fitness, foraging, and reproduction, increasing the effects of diseases like chytridiomycosis and contributing to the decline of many species[56,57,58,59,60,61,62]. The sampled *A. maraguapensis*, a critically endangered local species, experienced severe effects from mural and cavity myositis associated with encysted Nematoda larvae. The considerable body or cavity volume occupied by helminths (above 50%) in this small-bodied species exacerbates the physical impact, accelerating the reduction of its population.

Few viral diseases in amphibians have been described and reported. Ranaviruses (Iridoviridae) are emerging pathogens linked to mass mortality events in both wild and captive amphibian populations[63]. Due to their significant impact on the global decline of amphibians, the World Organization for Animal Health has included Ranaviruses on the notifiable diseases list[64]. Acute mortality outbreaks primarily affect larvae and metamorphs, presenting clinical symptoms such as lethargy, weakness, erratic swimming, anorexia, tegumentary changes, edema, and external hemorrhages. However, depending on the individual's immunocompetence and virulence of the agent, adults may also exhibit similar or milder symptoms[65]. The principal pathological mechanism of Ranavirus involves the invasion of endothelial cells, leading to cell death by inhibiting DNA, RNA, and protein synthesis[66]. Commonly observed pathological findings include diffuse hemorrhages and tissue necrosis, particularly affecting the liver, spleen, and kidneys—including their hematopoietic and lymphoid tissues—as well as skin ulcerations[65]. The presence of basophilic or amphophilic intracytoplasmic inclusions associated with necrosis and endothelial damage is highly indicative of Ranavirus infection, although their visualization can be challenging in necrotic tissues under conventional histopathological examination[65].

These findings underscore the necessity for an epizootiological investigation using gold-standard diagnostic methods to confirm Ranavirus infection. A previous case[67] involved a wild adult male *L. vastus* from an urban reserve in Fortaleza city, presenting multiple necrotic tissues with amphophilic intracytoplasmic inclusions in hepatocytes, splenocytes, and tubular cells, strongly suggesting chronic Ranavirus infection[65].

Moreover, 5 out of 27 cases with inconclusive etiology from the Aiuaba reserve exhibited similar inflammatory and vascular alterations: livers with moderate to diffuse melanomacrophage hyperplasia, moderate multifocal congested sinusoids, and central vessels, mild to moderate multifocal ballooning, and moderate multifocal hematopoiesis; moderate to marked multifocal granulomatous splenitis, with potential hypertensive reflexes causing congestive liver and kidney diseases. One of these five individuals displayed diffusely apoptotic lymphocytes alongside the previously mentioned hepatic and splenic changes. Given the morphology and frequency of these findings within the same geographic area, they suggest a viral infection[65], although intracellular inclusion bodies were not observed.

Mycobacteriosis in amphibians is commonly linked with macroscopic cutaneous or visceral granulomatous lesions, with reported frequencies ranging from 0.76% to 100%[23,68,69]. In the granulomas examined in this study, occasional epithelioid macrophages and lymphocytes were present, but not giant cells. The morphology was generally consistent with immune granulomas in the healing phase, characterized by granular material encapsulated by fibrous tissue, variable caseation, and occasional central calcification. Ackerman[70] noted that mycobacteria and their antigens are sparse within granulomas, which means they are seldom stained by classic or modified Ziehl-Neelsen techniques. Additionally, bacterial walls may lose staining characteristics in persistent or latent infections[71], and histological processing involving formaldehyde fixation and xylene clearing can reduce staining sensitivity, typically marking only 3 to 10% of bacilli in samples[72].

Speculation centered on whether the diffuse magenta marking within the granulomas was artifactual or represented expanded mycobacterial antigens in macrophages and necrotic tissue, a phenomenon that has been previously observed and quantified experimentally[73]. Given the similar granuloma morphology and epizootiological history of *M. gordonae* infection in frogs from southern Ceará State[23], and considering the negative results for PAS and Grocott staining (which excluded fungal granulomas), these five cases are also presumed to represent an etiological diagnosis of visceral mycobacteriosis. This condition has been documented in amphibians for over a century in Europe[74,75,76,77] and for several decades in Brazil[78,79,80], with reported morphology consistent with current findings.

Atypical mycobacteria, including *M. gordonae*, which has been previously detected in anurans from Ceará[23], are usually considered ubiquitous and non-pathogenic among several other non-tuberculous Mycobacteria. However, they have been associated with opportunistic infections in immunocompromised humans[81]. In captive animals, stressful conditions may trigger outbreaks with exuberant lesions[82]. Conversely, a previous case of marked systemic multifocal granulomatosis with acid-fast bacilli positive for ZN staining likely indicated an infection from the *M. tuberculosis* group (Braga, unpublished data), which is a significant zoonotic concern.

Prey-predator interactions are often the primary cause of traumatic injuries in frogs[83,84]. In Panama, researchers observed that the most common injury among a sample of *Dendrobates auratus* was digit amputation, occurring in 89.9% (532/592) of cases, followed by less frequent injuries such as limb fractures, limb amputations, and ocular enucleation[85]. The findings from the current study reveal musculoskeletal injuries in CHUFC specimens, including amputations of hands, feet, and entire legs, which left the radius-ulna and tibia bones exposed at their ends with adjacent soft tissues healed. These alterations suggest predation attempts and could have increased the vulnerability of these individuals to manual capture during subsequent research expeditions.

Terrestrial ectothermic animals can develop metabolic disorders that result in the mineralization of joints, organs, and soft tissues due to the deposition of nitrogenous crystals (uric gout) or calcium (pseudogout or calcinosis)[86]. While a few reports exist concerning sea turtles[51], there are none for terrestrial ectothermic wildlife. Such conditions are often linked to dietary imbalances in captive animals. For instance, calcinosis circumscripta was observed in Lithobates (syn. Rana) catesbeianus larvae near a sewage treatment station[87]. Some necropsy findings lacked previous clinical histories, necessitating complementary tests to distinguish between gout and calcinosis. At a veterinary conference, three cases were briefly presented where electronic and polarized microscopy confirmed a diagnosis of calcinosis/ silicosis. These cases introduced a pathophysiology hypothesis involving chronic ingestion of silicate-based agricultural additives, which led to hypomagnesemia and hypercalcemia, culminating in chronic renal failure with subsequent calcium deposition in soft tissues and organs (Braga, unpublished data). The current study includes another similar case from the same location.

Spontaneous tumors in amphibians have been documented since 1868[88]. A recent review identified 24 benign and 26 malignant tumors across 28 amphibian species (18 frogs and 10 caudates), including chromatophoromas (18%), lymphomas and leukemias (12%), and papillomas (10%)[89]. Twenty-four percent of the reported cases underwent surgical treatment, with survival ranging from 0.8 to 46 months post-operation. The review also noted two cases of lipomas, which are distinct from the fibrolipoma described in the current study due to the absence of fibrous bands around the adipose tissue nodules. No spontaneous cases of hepatocellular carcinoma have been recorded in amphibians, suggesting that this type of neoplasm is either rare or previously unreported. However, experimental research involving the subcutaneous injection of diethylstilbestrol in *Rana temporaria* resulted in 7/38 cases of hematopoietic neoplasia and 2/38 cases of undifferentiated hepatocellular carcinoma[90], suggesting that environmental xenobiotics may trigger such neoplasms. In the current study, the encysted nematodes found within the tumor could be linked to chronic inflammation and subsequent carcinogenesis. Trematoda helminths are often associated with liver carcinogenesis[91]; a recent hypothesis suggests that Nematodes produce transforming growth factor beta (TGF-β) to modulate the host's immune response in their favor, which might also promote the proliferation of neoplastic cells despite the typical negative regulation of antitumor immunity[92].

The unilateral ocular alteration observed in an *L. vastus* specimen could not be definitively classified due to the uncertainty surrounding its etiology. While it could be traumatic in nature, animal oculopathies are known to manifest symptoms such as retinal detachment and atrophy, often linked to viral infections[93]. For instance, in the US, a wild metamorphic specimen of *L. catesbeianus* exhibited unilateral ocular malformation characterized by reduced globe volume, scleral fibrosis, and granulomas, which were associated with viral particles of Ranavirus (FV-3)[94]. Although retinopathies are rare in amphibians, there have been some reports of retinal detachment due to acute necrosis associated with dermatosepticemia caused by gram-negative bacteria in *ex-situ* specimens[95,96]. In humans, viral retinopathies are well-documented, typically resulting from direct tissue damage or immune complexes related to Herpesvirus and Rubivirus[97]. Similar mechanisms might apply to Ranavirus and related viruses, which preferentially replicate in epithelial, endothelial, and lymphoid cells and can cause fibrinoid vasculitis in various organs[98]. Additionally, infections by Iridoviridae and other viruses may lead to the deposition of immunocomplexes in the vasculature of target organs, contributing to overall morbidity[99]. Furthermore, these viral interactions may trigger retinal autoimmunity[100].

5. Conclusion

Necropsy proves to be an essential tool for monitoring the health of anurans in Ceará State, Brazil. Postmortem examinations are crucial in identifying various diseases in specimens that exhibit no clinical or externally detectable signs. Examining specimens from scientific collections is particularly important as these collections serve as repositories of health information about the local fauna. Lesions suggestive of Ranavirus infection were noted, underscoring the need for further etiological diagnosis. A potential involvement of atypical *Mycobacteria* is particularly concerning given their unknown zoonotic potential. Additionally, significant parasitic lesions were identified, raising concerns about their potential impact on the fitness and ecological roles of these species. These findings place Ceará on the distribution map for significant diseases affecting anurofauna, highlighting the need for ongoing monitoring.

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: RR Braga. *Data curation*: RR Braga, DP de Castro, DC Lima and CR Oliveira. *Formal Analysis*: RR Braga. *Funding Acquisition*: DC Lima and RW Ávila. *Investigation*: RR Braga, DP de Castro and DC Lima. *Methodology*: RR Braga. *Project Administration*: RR Braga. *Writing (original draft)*: RR Braga. *Writing (review and editing)*: RR Braga, DP de Castro, DC Lima, CR Oliveira and RW Ávila.

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References

1. Frost D. Amphibian species of the world [Internet]. Amphibian Species of the World 6.1, an Online Reference. [cited 2023 Mar 12]. Available from: https://amphibiansoftheworld.amnh.org/

2. Wells KD. The Ecology & Behavior of Amphibians. Chicago: The University of Chicago Press; 2007.

3. Duellman WE, Trueb L. Biology of Amphibians. New York: McGraw-Hill; 1986.

4. Nunes-de-Almeida CHL, Haddad CFB, Toledo LF. A revised classification of the amphibian reproductive modes. Salamandra. 2021;57(3):413-427. Available from: https://www.salamandra-journal.com/index.php/contents/2021-vol-57/2054-nunes-de-almeida-c-h-l-c-f-b-haddad-l-f-toledo-1/file

5. Thibaudeau G, Altig R. Endotrophic Anurans. In: McDiarmic R, Altig R, editors. Tadpoles: the biology of anuran larvae. Chicago: The University of Chicago Press; 1999. p. 170-80.

6. Harfoot MBJ, Johnston A, Balmford A, Burgess ND, Butchart SHM, Dias MP, et al. Using the IUCN Red List to map threats to terrestrial vertebrates at global scale. Nature Ecology & Evolution. 2021;5:1510-1519. Available from: https://doi.org/10.1038/s41559-021-01542-9

7. Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R. Emerging Infectious Diseases and Amphibian Population Declines. Emerging Infectious Diseases. 1999;5(6):735-748. Available from: https://doi.org/10.3201/eid0506.990601

8. Densmore CL, Green DE. Diseases of amphibians. ILAR Journal. 2007;48(3):235-54. Available from: https://doi.org/10.1093/ilar.48.3.235

9. Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, et al. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science. 2019;363(6434):1459-1463. Available from: https://doi.org/10.1126/science.aav0379

10. Carvalho T, Becker CG, Toledo LF. Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. Proceedings of the Royal Society B: Biological Sciences. 2017;284(1848):20162254. Available from: http://dx.doi.org/10.1098/rspb.2016.2254

11. Toledo LF, Britto FB, Araújo O, Giasson L, Haddad C. The occurrence of *Batrachochytrium dendrobatidis* in Brazil and the inclusion of 17 new cases of infection. South American Journal of Herpetology. 2006;1(3):185-91. Available from: https://doi.org/10.2994/1808-9798(2006)1[185:TOOBDI]2.0.CO;2

12. Ruggeri J, Ribeiro LP, Pontes MR, Toffolo C, Candido M, Carriero MM, et al. Discovery of Wild Amphibians Infected with Ranavirus in Brazil. Journal of Wildlife Diseases. 2019;55(4):897-902. Available from: https://doi.org/10.7589/2018-09-224

13. Herczeg D, Ujszegi J, Kásler A, Holly D, Hettyey A. Host–multiparasite interactions in amphibians: a review. Parasites & Vectors. 2021;14(1):296. Available from: https://doi.org/10.1186/s13071-021-04796-1

14. Pessier AP. Amphibia. In: McAloose D, Leger JS, Terio KA, editors. Pathology of wildlife and zoo animals. London: Academic Press an imprint of Elsevier; 2018. p.919-950.

15. Darzins E. The epizootic of tuberculosis among the Gias in Bahia. Acta Tuberculosea Scandinavica. 1952;26(1-2):170-174. Available from: https://pubmed.ncbi.nlm.nih.gov/14952353/

16. Bartralot R, Pujol RM, García-Patos V, Sitjas D, Martín-Casabona N, Coll P, et al. Cutaneous infections due to nontuberculous mycobacteria: histopathological review of 28 cases. Comparative study between lesions observed in immunosuppressed patients and normal hosts. Journal of Cutaneous Pathology. 2000;27(3):124-129. Available from: https://doi.org/10.1034/j.1600-0560.2000.027003124.x

17. Cassiano-Lima D, Ávila RW, Castro DP, Roberto IJ, Borges-Nojosa DM. Lista de anfíbios do Ceará [Internet]. SEMA-CE. 2021 [cited 2023 Mar 10]. Available from: https://www.sema.ce.gov.br/fauna-do-ceara/vertebrados/anfibios/

18. Roberto IJ, Loebmann D. Composition, distribution patterns, and conservation priority areas for the herpetofauna of the state of Ceará, northeastern Brazil. Salamandra. 2016;52(2):134-152. Available from: https://www.salamandra-journal.com/index.php/contents/2016-vol-52/569-roberto-i-j-d-loebmann/file

19. Brasil. Ministério do Meio Ambiente. Gabinete do Ministro. Portaria no 148. Atualização da Lista Nacional de Espécies Ameaçadas de Extinção Brasília, DF; 2022. Portuguese. Available from: https://www.icmbio.gov.br/cepsul/images/stories/legislacao/Portaria/2020/P_mma_148_2022_altera_anexos_P_mma_443_444_445_2014_atualiza_especies_ameacadas_extincao.pdf

20. Preuss JF, Lambertini C, Leite D da S, Toledo LF, Lucas EM. *Batrachochytrium dendrobatidis* in near threatened and endangered amphibians in the southern Brazilian Atlantic Forest. North Western Journal of Zoology. 2015;11(2):360-362. Available from: https://biozoojournals.ro/nwjz/content/v11n2/nwjz_142504_Preuss.pdf

21. Benício RA, Carvalho T, Barbosa MD, Costa J de, da Silva FC, Fonseca MG. Worrying news for Brazilian Caatinga: Prevalence of *Batrachochytrium dendrobatidis* in Amphibians. Tropical Conservation Science. 2019;12:194008291989262. Available from: http://dx.doi.org/10.1177/1940082919892626

22. Carnaval AC, Puschendorf R, Peixoto OL, Verdade VK, Rodrigues MT. Amphibian Chytrid fungus broadly distributed in the Brazilian Atlantic Rain Forest. EcoHealth. 2006;3(1):41-48. Available from: http://dx.doi.org/10.1007/s10393-005-0008-2

23. Morais D, Rodrigues M, Ávila R, da Silva R. Visceral mycobacteriosis in amphibians from the Brazilian Caatinga region. Diseases of Aquatic Organisms. 2021;145(34):139-144. Available from: https://doi.org/10.3354/dao03604

24. Bassini-Silva R, Huang-Bastos M, Morais DH, Alcantara EP, Ávila RW, Welbourn C, et al. A new species of *Hannemania* oudemans, 1911 (Trombidiformes: Leeuwenhoekiidae) from Brazil. Journal of Natural History. 2021;55(19-20):1277-1287. Available from: https://doi.org/10.1080/00222933.2021.1944687

25. Lins AGS, Aguiar A, Morais DH, Firmino da Silva LA, Ávila RW, Silva RJ. Helminth fauna of *Leptodactylus syphax* (Anura: Leptodactylidae) from Caatinga biome, northeastern Brazil. Revista Brasileira de Parasitologia Veterinária. 2017;(1):74-80. Available from: https://doi.org/10.1590/S1984-29612017013

26. Mascarenhas W, Oliveira CR, Benício RA, Ávila RW, Ribeiro SC. Nematodes of *Proceratophrys ararype* (Anura: Odontophrynidae), an endemic frog from the Araripe Plateau, northeastern Brazil. Biota Neotropica. 2021;21(3): e20201164. Available from: https://doi.org/10.1590/1676-0611-BN-2020-1164

27. Oliveira CR, Ávila RW, Morais DH. Helminths Associated with Three Physalaemus Species (Anura: Leptodactylidae) from Caatinga Biome, Brazil. Acta Parasitologica. 2019;64(1):205-212. Available from: https://doi.org/10.2478/s11686-018-00022-8

28. Machado M, Castro MB, Gimeno EJ, Barros SS, Riet-Correa F. Enzootic calcinosis in ruminants: A review. Toxicon. 2020 Nov;187:1–9. Available from: https://doi.org/10.1016/j.toxicon.2020.08.009

29. Wagener MG, Lehmbecker A, Bühler M, Wilkens M, Punsmann T, Ganter M. Calcinosis in a roe deer fawn (*Capreolus capreolus*) in Northern Germany. BMC Veterinary Research. 2020;16(1):406. Available from: https://doi.org/10.1186/s12917-020-02615-w

30. Chatigny F, Kamunde C, Creighton CM, Stevens ED. Uses and doses of local anesthetics in fish, amphibians, and reptiles. Journal of the American Association for Laboratory Animal Science. 2017;56(3):244-253. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28378704

31. Brasil. Resolução n°1000 de 11 de Maio de 2012. [Internet]. Brasília: Conselho Federal de Medicina Veterinária, [Cited 2022 Set 18]. Portuguese. Available from: http://ts.cfmv.gov.br/manual/arquivos/resolucao/1000.pdf

32. Brasil. Resolução No 55, de 5 de Outubro de 2022. Diretriz Brasileira para o Cuidado e a Utilização de Animais em Atividades de Ensino ou de Pesquisa Científica. [Internet]. Conselho Nacional de Controle de Experimentação Animal, [Cited 2022 Dec 15]. Portuguese. Available from: https://www.gov.br/mcti/pt-br/composicao/conselhos/concea/arquivos/arquivo/legislacao/resolucao-normativa-no-55-de-5-de-outubro-de-2022.pdf

33. Campbell TC. Exotic Animal Hematology and Cytology. 4th ed. New York: John Wiley & Sons, Ltd; 2015. 402 p.

34. Pessier AP, Pinkerton M. Practical gross necropsy of amphibians. Seminars in Avian and Exotic Pet Medicine. 2003;12(2):81-88. Available from: https://doi.org/10.1053/saep.2003.127884

35. Tadrous PJ. Diagnostic Criteria Handbook in Histopathology: a surgical pathology vade mecum. 1st ed. West Sussex: John Wiley & Sons, Ltd; 2007. 422 p.

36. Azulay R, Andrade L. Demonstration of *Mycobacterium leprae* in sections in 532 cases of leprosy: comparative study between the Ziehl-Klingmuller and the Wade-Fite techniques. International Journal of Leprosy. 1954;22(2):195-199. Available from: https://pubmed.ncbi.nlm.nih.gov/13211040/

37. Greenwood N, Fox H. A comparison of methods for staining tubercle bacilli in histological sections. Journal of Clinical Pathology. 1973;26(4):253-257. Available from: https://doi.org/10.1136/jcp.26.4.253

38. R Core Team. R: A language and environment for statistical computing; 2022. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/.

39. Roberto IJ, Loebmann D. Composition, distribution patterns, and conservation priority areas for the herpetofauna of the state of Ceará, northeastern Brazil. Salamandra. 2016;52(2):134-152. Available from: https://www.salamandra-journal.com/index.php/contents/2016-vol-52/569-roberto-i-j-d-loebmann/file

40. Borges-Leite MJ, Mota-Rodrigues JF, Borges-Nojosa DM. Herpetofauna of a coastal region of northeastern Brazil. Herpetology Notes. 2014;7:405-413. Available from: https://www.seh-herpetology.org/journals/herpetology-notes/back-issues/volume-7-2014

41. Castro DP, Mângia S, Magalhães F de M, Röhr DL, Camurugi F, Silveira-Filho RR da, et al. Herpetofauna of protected areas in the Caatinga VI: the Ubajara National Park, Ceará, Brazil. Herpetology Notes. 2019;12:727-742. Available from: https://www.biotaxa.org/hn/article/view/31446

42. Castro DP, Mota-Rodrigues JF, Cassiano-Lima D, Borges-Nojosa DM. Composition and diversity of anurans from rock outcrops in the Caatinga Biome, Brazil. Herpetology Notes. 2018;11:189-195. Available from: https://www.biotaxa.org/hn/article/view/23510

43. Ribeiro SC, Roberto IJ, Sales DL, Ávila RW, Almeida WO. Amphibians and reptiles from the Araripe bioregion, northeastern Brazil. Salamandra. 2012;48(3):133-146. Available from: https://www.salamandra-journal.com/index.php/contents/2012-vol-48/292-ribeiro-s-c-i-j-roberto-d-l-sales-r-w-avila-w-o-almeida/file

44. Magalhães FM, Lyra ML, Carvalho TR, Baldo D, Brusquetti F, Burella P, et al. Taxonomic Review of South American Butter Frogs: Phylogeny, Geographic Patterns, and Species Delimitation in the *Leptodactylus latrans* Species Group (Anura: Leptodactylidae). Herpetological Monographs. 2020;34(1):131-177. Available from: https://doi.org/10.1655/0733-1347-31.4.131

45. Chaves MF, Moura GJB, Baptista JS, Dantas AP, Teixeira VW, Teixeira ÁAC. Environmental and abiotic factors interfere in *Leptodactylus macrosternum* (Anura, Leptodactylidae) reproduction despite no changes in sexual hormones levels. Research, Society and Development. 2021;10(8):e46110817627. Available from: https://doi.org/10.33448/rsd-v10i8.17627

46. Braga RR, Gondim PM, Matushima ER. Histopathology of Endocrine Organs of Miranda's White-Lipped Frogs (*Leptodactylus macrosternum*) from Cultivated and Non-Cultivated Regions in Semi-Arid Northeastern Brazil. Journal of Comparative Pathology. 2022;192:1-10. Available from: https://doi.org/10.1016/j.jcpa.2021.12.002

47. Braga RR, Gondim PM, Pereira RM, Batista BL, Matushima ER. *Leptodactylus macrosternum* (Anura: Leptodactylidae) as a bioindicator of potentially toxic chemical elements in irrigated perimeters in northeastern Brazil. Environmental Chemistry and Ecotoxicology. 2022;4:124-131. Available from: https://doi.org/10.1016/j.enceco.2022.02.003

48. Alvarado-Rybak M, Valenzuela-Sánchez A, Cevidanes A, Peñafiel-Ricaurte A, Uribe-Rivera DE, Flores E, et al. High prevalence of chigger mite infection in a forest-specialist frog with evidence of parasite-related granulomatous myositis. Parasitology Research. 2018;117(5):1643-1646. Available from: https://doi.org/10.1007/s00436-018-5822-x

49. Aisien MSO, Uwagbae M, Edo-Taiwo O, Imasuen AA, Ovwah E. Pattern of parasitic infections in anurans from a mangrove community of the Niger Delta, Nigeria. Zool. 2015;13(December):50–5. Available from: https://www.ajol.info/index.php/tzool/article/view/142143/131882#:~:text=oxyrynchus.,%25%20and%20nematodes%2C%2028.96%25.

50. Umberger CM, de Buron I, Roumillat WA, McElroy EJ. Effects of a muscle‐infecting parasitic nematode on the locomotor performance of their fish host. J Fish Biol. 2013 Apr 13;82(4):1250–8. Available from: https://doi.org/10.1111/jfb.12061

51. Kölle P, Hoffman R. Incidence of nephropathies in European Tortoises. Proceedings of the Association of Reptiles and Amphibians Veterinarians. 2002;33-35.

52. Galindo GM, Rodrigues RA, Marcondes SF, Soares P, Tavares LER, Fernandes CE. Morphological and morphometric features of nematode-cysts in *Gymnotus inaequilabiatus* liver in the Brazilian Pantanal. Revista Brasileira de Parasitologia Veterinária. 2017;26(3):285-291. Available from: https://doi.org/10.1590/S1984-29612017044

53. Melo FTV, Melo CSB, Nascimento LCS, Giese EG, Furtado AP, Santos JN. Morphological characterization of *Eustrongylides* sp. larvae (Nematoda, Dioctophymatoidea) parasite of *Rhinella marina* (Amphibia: Bufonidae) from Eastern Amazonia. Revista Brasileira de Parasitologia Veterinária. 2016;25(2):235-239. Available from: https://doi.org/10.1590/S1984-29612016024

54. Jones HI. Pathology associated with Physalopterid larvae (Nematoda: Spiruridae) in the gastric tissues of Australian reptiles. Journal of Wildlife Diseases. 1995;31(3):299-306. Available from: https://doi.org/10.7589/0090-3558-31.3.299

55. Juan-Sallés C, Almagro V, Carbonell L, Valls X, Montesinos A, Fernández-Bellon H. Enfermedades infecciosas y parasitarias en anfibios en cautividad: estudio retrospectivo de 131 pacientes. Clínica Veterinaria de Pequeños Animales. 2020;40(1):15-27. Available from: https://www.clinvetpeqanim.com/img/pdf/2025110247.pdf

56. Goater CP, Semlitsch RD, Bernasconi MV. Effects of Body Size and Parasite Infection on the Locomotory Performance of Juvenile Toads, *Bufo bufo*. Oikos. 1993;66(1):129-136. Available from: https://doi.org/10.2307/3545205

57. Wetsch O, Strasburg M, McQuigg J, Boone MD. Is overwintering mortality driving enigmatic declines? Evaluating the impacts of trematodes and the amphibian chytrid fungus on an anuran from hatching through overwintering. PLOS ONE. 2022;17(1):e0262561. Available from: https://doi.org/10.1371/journal.pone.0262561

58. Sanil NK, Asokan PK, John L, Vijayan KK. Pathological manifestations of the acanthocephalan parasite, *Tenuiproboscis* sp. in the mangrove red snapper (*Lutjanus argentimaculatus*) (Forsskål, 1775), a candidate species for aquaculture from Southern India. Aquaculture. 2011;310(3-4):259-266. Available from: https://doi.org/10.1016/j.aquaculture.2010.10.027

59. Choi C-J, Lee H-J, Go J-H, Park Y-K, Chai J-Y, Seo M. Extraintestinal Migration of *Centrorhynchus* sp. (Acanthocephala: Centrorrhynchidae) in Experimentally Infected Rats. The Korean Journal of Parasitology. 2010;48(2):139-143. Available from: https://doi.org/10.3347/kjp.2010.48.2.139

60. Stutz WE, Blaustein AR, Briggs CJ, Hoverman JT, Rohr JR, Johnson PTJ. Using multi-response models to investigate pathogen coinfections across scales: Insights from emerging diseases of amphibians. Methods in Ecology and Evolution. 2017;9(4):1109-1120. Available from: https://doi.org/10.1111/2041-210X.12938

61. Campião KM, Ribas AC de A, Morais DH, Silva RJ da, Tavares LER. How Many Parasites Species a Frog Might Have? Determinants of Parasite Diversity in South American Anurans. PLOS ONE. 2015;10(10):e0140577. Available from: https://doi.org/10.1371/journal.pone.0140577

62. Szuroczki D, Richardson JML. The role of trematode parasites in larval anuran communities: an aquatic ecologist's guide to the major players. Oecologia. 2009;161(2):371-385. Available from: https://doi.org/10.1007/s00442-009-1388-8

63. Miller D, Gray M, Storfer A. Ecopathology of Ranaviruses Infecting Amphibians. Viruses. 2011 Nov 22;3(11):2351–73.

64. World Organisation for Animal Health. Infection with Ranavirus [Internet]. Manual of Diagnostic Tests for Aquatic Animals. 2022 [cited 2022 Nov 1]. Available from: https://www.woah.org/en/disease/ranavirosis/

65. Miller DL, Pessier AP, Hick P, Whittington RJ. Comparative Pathology of Ranaviruses and Diagnostic Techniques. In: Gray MJ, Chinchar VG, editors. Ranaviruses lethal pathogens of ectothermic vertebrates. Cham: Springer International Publishing; 2015. p. 171-208.

66. Jancovich JK, Qin Q, Zhang Q, Chinchar VG. Ranavirus Replication: Molecular, Cellular, and Immunological Events. In: Gray MJ, Chinchar VG, editors. Ranaviruses lethal pathogens of ectothermic vertebrates. Cham: Springer International Publishing; 2015. p. 105-140.

67. Braga RR, Eleutério BKN, Lima RCS, Quirino TF. Marked systemic necrotizing disease in a *Leptodactylus vastus* (Anura: Leptodactylidae) from an urban reserve in northeastern Brazil. Brazilian Journal of Veterinary Pathology, 2023;16(3):203-207. Available from: DOI: https://10.24070/bjvp.1983-0246.v16i3p203-207

68. Fremont-Rahl JJ, Ek C, Williamson HR, Small PLC, Fox JG, Muthupalani S. *Mycobacterium liflandii* Outbreak in a Research Colony of *Xenopus* (Silurana) tropicalis Frogs. Veterinary Pathology. 2011;48(4):856-867. Available from: https://doi.org/10.1177/0300985810388520

69. Milnes EL, Delnatte P, Lentini A, May K, Ma J, Jamieson FB, et al. Mycobacteriosis in a Zoo Population of Chinese Gliding Frogs (*Rhacophorus dennysi*) Due to *Mycobacterium marinum*. Journal of Herpetological Medicine and Surgery. 2020;30(1):14-20. Available from: https://doi.org/10.5818/19-03-186.2

70. Ackermann M. Inflammation and Healing. In: Zachary JF, editor. Pathologic basis of veterinary diseases. 7th ed. Saint Louis: Elsevier; 2022. p. 104-170.

71. Seiler P, Ulrichs T, Bandermann S, Pradl L, Jörg S, Krenn V, et al. Cell-Wall Alterations as an Attribute of *Mycobacterium tuberculosis* in Latent Infection. The Journal of Infectious Diseases. 2003;188(9):1326-1331. Available from: https://doi.org/10.1086/378563

72. Fukunaga H, Murakami T, Gondo T, Sugi K, Ishihara T. Sensitivity of Acid-Fast Staining for Mycobacterium tuberculosis in Formalin-fixed Tissue. American Journal of Respiratory and Critical Care Medicine. 2002;166(7):994-997. Available from: https://doi.org/10.1164/rccm.2111028

73. Riaz SM, Bjune GA, Wiker HG, Sviland L, Mustafa T. Mycobacterial antigens accumulation in foamy macrophages in murine pulmonary tuberculosis lesions: Association with necrosis and making of cavities. Scandinavian Journal of Immunology. 2020;91(4): e12866. Available from: https://doi.org/10.1111/sji.12866

74. Elkan E. Some interesting pathological cases in amphibians. Proceedings of the Zoological Society of London. 1960;134(2):275-296. Available from: https://doi.org/10.1111/j.1469-7998.1960.tb05593.x

75. Griffith A. Tuberculosis in cold-blooded animals. In: A system of bacteriology. London: Medical Research Council; 1930. p. 326-332.

76. Küster E. Über Kaltblütertuberkulose. Munchener medizinische Wochenschrift. 1905;52:57-59 (cited by Barros et al., 1988).

77. Lichtenstein S. Ein Fall von spontaner Froschtuberkulose. Centralblatt für Bakteriologie, Parasitenkunde. 1920;85:249-252 (cited by Barros et al., 1988).

78. Barros G, Langenegger C, Langenegger J, Peixoto P. Surto de micobacteriose em criação de rãs (*Rana catesbeiana*) causado por *Mycobacterium marinum*. Pesquisa Veterinária Brasileira. 1988;8(3/4):75-80. Portuguese.

79. Darzins E. The epizootic of tuberculosis among the Gias in Bahia. Acta Tuberculosea Scandinavica. 1952;26(1-2):170-174. Available from: https://pubmed.ncbi.nlm.nih.gov/14952353/

80. Ferreira R, Fonseca L de S, Afonso AM, da Silva MG, Saad MH, Lilenbaum W. A report of mycobacteriosis caused by *Mycobacterium marinum* in bullfrogs (*Rana catesbeiana*). The Veterinary Journal. 2006;171(1):177-180. Available from: https://doi.org/10.1016/j.tvjl.2004.08.018

81. Bartralot R, Pujol RM, García-Patos V, Sitjas D, Martín-Casabona N, Coll P, et al. Cutaneous infections due to nontuberculous mycobacteria: histopathological review of 28 cases. Comparative study between lesions observed in immunosuppressed patients and normal hosts. Journal of Cutaneous Pathology. 2000;27(3):124-129. Available from: https://doi.org/10.1034/j.1600-0560.2000.027003124.x

82. Sánchez-Morgado JM, Gallagher A, Johnson LK. *Mycobacterium gordonae* infection in a colony of African clawed frogs (*Xenopus tropicalis*). Laboratory Animals. 2009;43(3):300-303. Available from: https://doi.org/10.1258/la.2008.008035

83. Dubois A. Anomalies and mutations in natural populations of the *Rana esculenta* complex (Amphibia, Anura). Mitteilungen aus dem Museum für Naturkunde in Berlin Zoologisches Museum und Institut für Spezielle Zoologie [Berlin]. 1979;55(1):59-87.

84. Martof B. Factors influencing size and composition of populations of *Rana clamitans*. American Midland Naturalist. 1956;56(1):224-245. Available from: http://dx.doi.org/10.2307/2422457

85. Gray HM, Ouellet M, Green DM, Rand AS. Traumatic injuries in two neotropical frogs, *Dendrobates auratus* and *Physalaemus pustulosus*. Journal of Herpetology. 2002;36(1):117-121. Available from: http://dx.doi.org/10.1670/0022-1511(2002)036[0117:tiitnf]2.0.co;2

86. Orós J. Pseudogout. In: Divers SJ, Stahl SJ, editors. Mader's reptile and Amphibian Medicine and Surgery. St. Louis, MO: Elsevier; 2019. pp.1333-1334.

87. Keel MK, Ruiz AM, Fisk AT, Rumbeiha WK, Davis AK, Maerz JC. Soft-tissue mineralization of bullfrog larvae (*Rana catesbeiana*) at a wastewater treatment facility. Journal of Veterinary Diagnostic Investigation. 2010;22:655-660. Available from: https://doi.org/10.1177/104063871002200430

88. Balls M. Spontaneous Neoplasms in Amphibia: A Review and Descriptions of Six New Cases. Cancer Research. 1962;22(10):1142-1154. Available from: https://aacrjournals.org/cancerres/article/22/10/1142/474833/Spontaneous-Neoplasms-in-Amphibia-A-Review-and

89. Hopewell E, Harrison SH, Posey R, Duke EG, Troan B, Harrison T. Analysis of Published Amphibian Neoplasia Case Reports. Journal of Herpetological Medicine and Surgery. 2020;30(3):148-155. Available from: https://doi.org/10.5818/19-09-212.1

90. Khudolei V, Ermoshchenkov V. Carcinogenic action of diethylstilbestrol on frogs. Bulletin of Experimental Biology and Medicine. 1976;81:898-900. Available from: https://doi.org/10.1007/BF00803017

91. Abdel-Rahim AY. Parasitic Infections and Hepatic Neoplasia. Digestive Diseases. 2001;19(4):288-291. Available from: https://doi.org/10.1159/000050695

92. Maruszewska-Cheruiyot M, Stear MJ, Machcińska M, Donskow-Łysoniewska K. Importance of TGFβ in Cancer and Nematode Infection and Their Interaction—Opinion. Biomolecules. 2022;12(11):1572. Available from: https://doi.org/10.3390/biom12111572

93. Aroch I, Ofri R, Sutton GA. Ocular Manifestations of Systemic Diseases. Slatter's Fundamentals of Veterinary Ophthalmology. 2008;374-418. Available from: https://doi.org/10.1016/B978-072160561-6.50021-6

94. Burton EC, Miller DL, Styer EL, Gray MJ. Amphibian ocular malformation associated with frog virus 3. The Veterinary Journal. 2008;177(3):442-444. Available from: https://doi.org/10.1016/j.tvjl.2007.05.006

95. Brooks DE, Jacobson ER, Wolf ED, Clubb S, Gaskin JM. Panophthalmitis and otitis interna in fire-bellied toads. Journal of American Veterinary Medical Association. 1983;183(11):1198-201. Available from: https://pubmed.ncbi.nlm.nih.gov/6643232/

96. Olson ME, Gard S, Brown M, Hampton R, Morck DW. *Flavobacterium indologenes* infection in leopard frogs. Journal of American Veterinary Medical Association. 1992;201(11):1766-1770. Available from: https://pubmed.ncbi.nlm.nih.gov/1293124/

97. Weiter JJ, Roh S. Viral infections of the choroid and Retina. Infectious Disease Clinics of North America. 1992;6(4):875-891. Available from: https://pubmed.ncbi.nlm.nih.gov/1460268/

98. Forzán MJ, Jones KM, Ariel E, Whittington RJ, Wood J, Markham RJ, et al. Pathogenesis of Frog Virus 3 (*Ranavirus*, *Iridoviridae*) infection in wood frogs (*Rana sylvatica*). Veterinary Pathology. 2017;54(3):531-548. Available from: https://doi.org/10.1177/0300985816684929

99. Monini M, Ruggeri FM. Antigenic peptides of the Epizootic hematopoietic necrosis virus. Virology. 2002;297(1):8–18. Available from: https://doi.org/10.1006/viro.2002.1410

100. Hooks JJ, Detrick B, Nussenblatt R. Infections associated with retinal autoimmunity. Infection and Autoimmunity. 2004:691-700. Available from: https://doi.org/10.1016/B978-044451271-0.50054-5