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Livestock diseases in Northeastern Brazilian Amazon region: A survey of 448 tissues samples submitted for histopathological evaluation between 1997 and 2017

Enfermidades de animais de produção no nordeste da Amazônia: Um levantamento de 448 amostras de tecido encaminhadas para avaliação histopatológica entre 1997 e 2017

Enfermedades de los animals de granja en el noreste de la Amazonia: Un estudio de 448 muestras de tejido enviadas para evaluación histopatológica entre 1997 y 2017

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Abstract
The amazon river The Amazon River basin is known for its great variety of vegetation and animals, as well as for the prominent cattle ranching. However, Amazonia hot and humid natural climate is a suitable environment for the development and spread of diseases, posing a challenge for the livestock sector. This study aimed to study livestock diseases in the northeastern Brazilian Amazon Region though a survey of tissue samples submitted to histopathological. A retrospective study was conducted in the archives of the Anatomical Pathology Sector of the Federal Rural University of Rio de Janeiro, Brazil, between 1997 and 2017. It was included tissue of bovine, bubaline, equine, ovine, and caprine collected in the Northeastern Brazilian Amazon Region and submitted to histopathological evaluation. Data on species, histologic description, and diagnosis were retrieved from the pathological reports, and ancillary techniques were applied to elucidate inconclusive cases. Between January 1997 and July 2017, 448 tissue samples were submitted for histopathological evaluation. Infectious diseases comprised 31.5% (141/448) of all diagnoses, followed by mineral imbalances (16.5%), neoplasia (10.7%), and poisonous plants and related conditions (8.9%). Histochemical, immunohistochemical and molecular techniques proved seem to be useful to help elucidate inconclusive cases.
Keywords: Amazonia; Histology, Necropsy; Farm animals.

Resumo

A bacia do Rio Amazonas é conhecida pela grande variedade de vegetação e animais, bem como pelo expoente setor pecuarista. Contudo, o clima quente e úmido, natural da Amazônia, é um ambiente adequado para o desenvolvimento e propagação de doenças, representando um desafio para o setor pecuário. Objetivou-se com esse trabalho estudas as doenças de animais de produção no nordeste da região Amazônica por meio do levantamento de amostras de tecido submetidas à avaliação histopatológica. Um estudo retrospectivo foi conduzido nos arquivos do Setor de Anatomia Patológica da Universidade Federal Rural do Rio de Janeiro entre 1997 e 2017. Foram incluídos tecidos de bovinos, bubalinos, equinos, ovinos e caprinos coletados no nordeste da região Amazônica e encaminhados para avaliação histopatológica. Dados sobre a espécie, descrição histológica e diagnóstico foram levantados dos laudos patológicos, e exames complementares foram realizados na tentativa de elucidar casos anteriormente inconclusivos. Entre janeiro de 1997 e julho de 2017, 448 amostras de tecido foram submetidas para avaliação histopatológica. As doenças infecciosas representaram 31,5% (141/448) de todos os diagnósticos, seguidas por desequilíbrios minerais (16,5%), neoplasias (10,7%) e doenças causadas por plantas tóxicas (8,9%). Técnicas histoquímicas, imuno-histoquímicas e moleculares mostraram-se úteis para auxiliar na elucidação de casos inconclusivos.

Palavras-chave: Amazônia; Histologia; Necropsia; Animais de produção.

Resumen

La cuenca del Río Amazonas es conocida por su gran variedad de vegetación y animales, así como por su destacado sector ganadero. Sin embargo, el clima cálido y húmedo, característico de la Amazonía, crea un entorno propicio para el desarrollo y propagación de enfermedades, representando un desafío para el sector pecuario. Este trabajo tuvo como objetivo estudiar las enfermedades de los animales de producción en el noreste de la región Amazónica mediante el levantamiento de muestras de tejido sometidas a evaluación histopatológica. Se realizó un estudio retrospectivo en los archivos del Sector de Anatomía Patológica de la Universidad Federal Rural de Río de Janeiro entre 1997 y 2017. Se incluyeron tejidos de bovinos, búfalos, equinos, ovinos y caprinos recolectados en el noreste de la región Amazónica y remitidos para evaluación histopatológica. Se recopilaron datos sobre la especie, descripción histológica y diagnóstico de los informes patológicos, y se realizaron exámenes complementarios para intentar esclarecer casos previamente inconclusos. Entre enero de 1997 y julio de 2017, se sometieron 448 muestras de tejido para evaluación histopatológica. Las enfermedades infecciosas representaron el 31,5% (141/448) de todos los diagnósticos, seguidas de desequilibrios minerales (16,5%), neoplasias (10,7%) y enfermedades causadas por plantas tóxicas (8,9%). Las técnicas histoquímicas, inmunohistoquímicas y moleculares demostraron ser útiles para ayudar en la elucidación de casos inconclusos.

Palabras clave: Amazonía; Histología; Necropsia; Animales de producción.

1. Introduction

The Amazon River basin holds the largest rainforest in the world and a great variety of vegetation and animal species. The Brazilian Amazon Region is known for its high development and sustainable economic activities, with cattle ranching playing a major role. The vast pasturelands support huge herds of cattle, sustains the largest buffalo herd worldwide and generate over 700 million pounds annually with beef exportation (Freitas-Júnior, 2021).

Since livestock production has significant economic, environmental, and social implications in the Brazilian Amazon region, understanding its limiting factors is crucial, especially the diseases affecting these herds. The intrinsic biological diversity of Amazonia means that well-known diseases in other ecosystems present unique features in the Amazonian biome. Moreover, the hot and humid natural climate in Amazonia creates a suitable environment for vector-borne diseases, posing a significant challenge for the livestock sector (Vudriko et al., 2021).

Retrospective studies that retrieve data from laboratory archives are a valuable research methodology in several fields, including veterinary sciences. These cost-effective and time-efficient studies provide quick access to large amounts of data and allow for thorough analysis of the epidemiological, clinical, and morphological aspects of various diseases. Additionally, the availability of stored samples facilitates the use of ancillary techniques to elucidate or aid previously inconclusive or undetermined cases (Talari & Goyal, 2020).

In this sense, we aimed to study livestock diseases in the northeastern Brazilian Amazon Region though a survey of tissue samples submitted to histopathological evaluation between 1997 and 2017.

2. Methodology

We conducted a retrospective study in the archives of the Anatomical Pathology Sector (SAP) of the Federal Rural University of Rio de Janeiro following the STROBE guidelines as reviewed by Cuschieri (2019). We included all tissue samples of bovine, bubaline, equine, ovine, and caprine collected in an area of interest of the Federal Rural University of Pará and submitted to histopathological evaluation between March 1997 and December 2017. Samples submitted as part of experimental studies were excluded.

Patients' laboratory requisition forms and pathological reports were scanned to collect information regarding species, histologic description, and diagnosis of each case.

A histological revision was performed using slides stained with Hematoxylin and Eosin, and ancillary tests such as histochemistry, immunohistochemistry, and molecular techniques were used to aid inconclusive cases. The original morphological or etiological diagnosis was maintained when diagnosis could not be either confirmed or refuted by ancillary techniques.

Data was treated qualitatively. Diseases were grouped by species and etiology, and the results were expressed in charts and tables.

3. Results

Between January 1997 and July 2017, 448 tissue samples were submitted for histopathological evaluation: 37.9% (170/448) from bovine, 26.7% (120/448) from bubaline, 20.5% (92/448) from equid, 10.2 % (46/448) from ovine, and 4.4% (20/448) from caprine. Infectious diseases comprised 31.5% (141/448) of all diagnoses, followed by mineral imbalances (16.5%), neoplasia (10.7%), and poisonous plants and related conditions (8.9%). Samples in an advanced autolysis stage and yielding unspecific histologic lesions were considered inconclusive, totaling 35 cases (Table 1).

Table 1 - Absolute values of diseases diagnosed in livestock raised in northeastern Amazon region between 1997 and 2017, sorted by etiology.

Etiology/Animal species	Cattle	Buffalo	Equine	Sheep	Caprine	Total
Infectious diseases	42	38	54	4	3	141
Poisonous plants and related conditions	9	2	13	9	7	40
Mineral Imbalances	27	40	-	5	2	74
Poisoning by mineral and chemical elements	9	3	-	12	-	24
Neoplasia	21	15	10	-	2	48
Miscellaneous	38	19	10	14	5	86
Inconclusive	24	3	5	2	1	35
Total	170	120	92	46	20	448

Source: Authors.

Table 2 displays the infectious diseases diagnosed in our survey. Histologically, the case of rabies in cattle and horses showed perivascular lymphoplasmacytic encephalitis in the brain's white matter. Negri bodies were found within the cytoplasm of neurons in 11 cattle and five horses. The remaining cases were confirmed with the aid of immunohistochemistry.

Thirty and one pathological files of horses report equine infectious anemia as the presumptive diagnosis. Liver and spleen tissue samples presented an intense and multifocal to diffuse intracytoplasmic accumulation of golden brow granules (hemosiderosis). Additionally, a few cases presented pariacinar necrosis of the liver and discrete periportal infiltration by lymphocytes and macrophages.

In seventeen files there was a clinical history of chessy masses observed grossly in the lungs, liver, and intestine of cattle, buffaloes, and goats. Those samples yielded the classic image of tuberculosis granuloma - a granulomatous chronic inflammation with central necrosis and mineralization surrounded by lymphocytes and a fibrous capsule. We used the modified Zieh-Neelsen stain to identify alcohol acid-resistant bacilli within the lesions and confirm the mycobacterial infection.

Twelve cases of lymphoplasmacytic meningoencephalitis and polioencephalomalacia were associated with herpes virus infection. Six brain samples were positive for herpesvirus on the polymerase chain reaction - three bovine BOHV-5 and three EHV- 1. A brain sample from a cattle was negative on PCR but exhibited herpetic intracytoplasmatic inclusion bodies in the histological slides. The remaining cases were diagnosed based on histologic findings and clinical history.

Pythiosis was the only significant fungal infection in our study. Ulcerated and crater-like skin masses revealed fibrovascular connective and coagulative necrosis containing a diffuse inflammation of eosinophils, lymphocytes, and plasma cells. Negatively stained hyphae within the necrotic foci were readily highlighted with the Grocott methenamine silver stain.

A brief histology description of the infectious diseases we identified in the study can be appreciated in Table 3.

Table 2 - Absolute values of infectious diseases diagnosed in livestock raised in northeastern Amazon region between 1997 and 2017.

Disease/Animal species	***Cattle***	***Buffalo***	***Equine***	***Sheep***	***Caprine***	**Total**
Rabies	15	-	5	-	-	20
Herpesvirus infection	9	-	3	-	-	12
Botulism	5	-	-	-	-	5
Bovine Enzootic Leukosis	4	-	-	-	-	4
Salmonellosis	2	-	-	-	-	2
Actinobacylosis	1	-	-	-	-	1
Dermatophytosis	1	-	-	-	-	1
Dermatophilosis	1	-	-	-	-	1
Bovine Viral Diarrhea	1	1	-	-	-	2
Paratuberculosis	1	-	-	-	-	1
Tuberculosis	1	15	-	-	1	17
Bovine Syncytial Respiratory virus	1	1	-	-	-	2
Brucellosis	-	20	-	-	-	20
Malignant Catarrhal Fever	-	1	-	-	-	1
Equine Infectious Anemia	-	-	31	-	-	31
Pythiosis	-	-	9	-	-	9
Equine Encephalitis	-	-	5	-	-	5
Botryomycosis	-	-	1	-	-	1
Contagious Ecthyma	-	-		2	2	4
Caseous lymphadenitis	-	-		1	-	1
Phycomycosis				1	-	1
Total	42	38	54	4	3	141

Source: Authors.

Table 3 - Histologic findings of infectious diseases in livestock raised in northeastern Amazon region between 1997 and 2017.

Disease/Animal species	*Tissue*	*Histologic findings*
Rabies	Central Nervous System	Lymphoplasmacytic perivasculitis and meningitis. Intracytoplasmic eosinophilic Negri bodies.
Herpesvirus infection	Central Nervous System	Lymphoplasmacytic perivasculitis and meningitis. Laminar cortical necrosis of neurons; enlarged astrocytes; vacuolization and patchy regions in the parenchyma (polioencephalomalacia). Eventual astrocyte intranuclear eosinophilic viral bodies.
Botulism	Central Nervous System	Lack of microscopic findings. The diagnosis is based on exclusion of other neurological conditions that yield histologic lesions.
Bovine Enzootic Leukosis	Hearth, spleen, lymph nodes, kidneys, liver	Proliferation of atypical large lymphocytes that diffusely replaces the tissue's parenchyma (Lymphoma)
Salmonellosis	Liver, spleen, central nervous system	Central Nervous System: meningoencephalitis ranging from lymphoplasmacytic to purulent (abscesses) Spleen: pyogranulomatous splenitis Liver: fibrinoid to coagulative necrosis; mononuclear hepatitis rich in lymphocytes and plasm cells. Fibrinoid thrombosis
Actinobacylosis	Tongue	Multifocal to coalescent granulomatous inflammation with Splendore Hoepli phenomenon
Dermatophytosis	Skin	Hair shafts contain numerous ecto-and endothrix arthrospores and fungal hyphae. Fungal structures are readily seen with the aid of Period Acid Schiff Stain (PAS)
Dermatophylosis	Skin	Epidermis covered with a thick serocellular crust rich in degenerated neutrophils. The crust contains filamentous chains of gram-positive zoospores;
Bovine Viral Diarrhea	Intestine	Mucosal erosion, loss of villi structure, and collapse of the lamina propria; lamina propria expanded by lymphocytes, plasm cells, macrophages, and few neutrophils. Peyer's patches lymphocytes exhibiting severe karyorrhexis
Paratuberculosis	Intestine, lymph nodes, liver, lungs, kidneys	Granulomatous inflammation rich in epithelioid macrophages and Langham's giant cells. The lesions are more severe in the gastrointestinal tract. *Mycobacterius* paratuberculosis bacilli are numerous and easily identifiable with the aid of Ziehl-Neelsen stain.
Tuberculosis	Lungs, liver, lymph nodes, uterus, ovary,	Classic tuberculosis granuloma rich in epithelioid macrophages and Langshan's giant cells, with or without mineralization. Modified Ziehl-Neelsen stain is preferable to visualize Acid-alcohol-Resistant Bacilli in bovine and bubaline samples.
Bovine Syncytial Respiratory virus	Lungs	Interstitial pneumonia rich in syncytial cells. Eventual intracytoplasmic eosinophilic viral bodies.
Brucellosis	Placenta	Purulent necrotizing placentitis. Numerous bacterial colonies within the necrotic areas
Malignant Catarrhal Fever	Liver, Central Nervous System, spleen, lymph nodes, kidney	Lymphoplasmacytic perivascular infiltrates; fibrinoid arteritis
Equine Infectious Anemia	Spleen, liver, lymph nodes	Macrophages filled with intracytoplasmic brown golden granules (hemosiderosis)
Pythiosis	Skin	Masses are composed of severe infiltration by eosinophils, lymphocytes, and plasm cell with a necrotic center and arranged in a granuloma pattern (eosinophilic granuloma). Hyphae are visualized with the aid of Grocott stain.
Equine Encephalitis	Central Nervous System	Lymphoplasmacytic perivasculitis and meningitis;
Botryomycosis	Skin	Multifocal to coalescent pyogranulomatous inflammation and Splendore Hoepli phenomenon. Bacterial colonies visible within the granulomas;
Contagious Ecthyma	Skin and oral mucosa	Intraepidermal vesicles filled with neutrophils and cellular debris
Caseous lymphadenitis	Lymph nodes	Extensive caseous necrosis and granulomatous inflammation rich in giant cells and basophilic bacteria
Phycomycosis	Skin	Multifocal to coalescent pyogranulomatous inflammation and Splendore Hoepli phenomenon. Negative images of hyphae in the center of granulomas. Fungal structures are visible with Grocott stain

Source: Authors.

Histologic changes suggestive of mineral imbalances were recorded in 74 tissue samples; 27 from cattle, 40 from buffalo, five from sheep, and two from caprine (Table 4). Histologic changes associated with phosphorus, cobalt and copper deficiencies can be seen in the supplementary Table 5.

Table 4 - Absolute values of mineral imbalances diagnosed in livestock raised in northeastern Amazon region between 1997 and 2017.

Mineral/Animal species	Cattle	Buffalo	Equine	Sheep	Caprine	Total
Phosphorus	2	4	-	-	-	6
Copper	7	16	-	4	1	28
Cobalt	18	20	-	1	1	40
Total	27	40	-	5	2	74

Source: Authors.

Table 5 - Histologic findings of mineral imbalances in livestock raised in northeastern Amazon region between 1997 and 2017.

Mineral	Tissue	Histologic findings
Phosphorus	Bone	Cortical bone exhibits severe demineralization and bone trabeculae are bordered with osteoid tissue (Figure)
Copper	Spleen, lymph node, liver	Accumulation of macrophages filled with intracytoplasmic golden brow granules (hemosiderin)
Cobalt	Liver	Hepatocytes multifocally to diffusely filled with intracytoplasmic vacuoles (fatty degeneration)

Source: Authors.

Neoplastic diseases were our survey's third most common condition, only seconding infectious diseases and mineral imbalances. Forty-eight diagnoses were made - 21 in cattle, 15 in bubaline, 10 in horses, and two in goats (Table 6). The cases occurred isolated, and epithelial cutaneous malignancies prevailed - we confirmed 15 diagnoses of squamous cell carcinoma, followed by five cases of malignant melanotic melanoma. The entry "lymphoid neoplasia" comprised tumors rich in lymphocytes that could not be associated with the Bovine Leukemia Virus; we included this entity above in this study's "infectious diseases" section.

Table 6 - Absolute values of neoplastic diseases in livestock raised in northeastern Amazon region between 1997 and 2017.

Neoplasia/Animal species	*Cattle*	*Buffalo*	*Equine*	*Sheep*	*Caprine*	Total
Squamous Cell Carcinoma	9	2	3	-	1	15
Malignant melanotic melanoma	3	-	2	-		5
Fibrosarcoma	2	-	-	-		2
Neurofibroma	2	1	-	-		3
Ocular Carcinoma	1	-	-	-		1
Leiomyosarcoma	1	-	-	-		1
Lymphoid neoplasia	1	7	1	-		9
Papilloma	1	-	-	-		1
Sarcoma of unknow origin	1	-	-	-	1	2
Pheochromocytoma	-	2	-	-		2
Leiomyoma	-	1	-	-		1
Osteocondrosarcoma	-	1	-	-		1
Thecoma	-	1	-	-		1
Equine Sarcoid	-	-	3	-	-	3
Myxoid Sarcoma		-	1	-	-	1
Total	21	15	10	-	2	48

Source: Authors.

Clinical diseases and deaths associated with ingesting of poisonous plants in our study mainly occurred as outbreaks. Plants affecting the central nervous system were prevalent; we diagnosed nine cases consistent with poisoning by *Ipomoea*

asarifolia and six cases with degenerative histologic brain lesions suggestive of poisoning by *Ipomoea carnea* sub. *fistulosa* (Table 5).

The diagnosis of poisoning by *ipomoea asarifolia* was based on clinical and epidemiological findings associated with the lack of histologic lesions in the brain. According to the data retrieved from the clinical reports, the affected livestock was raised in paddocks infested with *Ipomoea asarifolia* and developed a tremorgenic syndrome following the ingestion of the plant.

Cases of sudden death due to ingestion of *Palicourea* and other cardiac plants were minimal in our study. One cattle presented degenerative changes in the kidney, seen as large intracytoplasmic vacuoles in cortical renal tubular epithelial cells (Table 6). According to clinical history, the bovine was raised in a pasture rich in *Palicourea juruana*.

Outbreaks of secondary (hepatic) photosensitization were recorded in livestock feeding with *Brachiaria* (*Urochloa*) sp. The diagnosis was based on the characteristic cutaneous lesions and the presence of foamy macrophages in the liver. One must notice, however, that foamy macrophages indicate the ingestion of *Brachiaria* and should not be used as standard criteria for signaling poisoning.

Microscopic features of the cases of poisoning by plants and related conditions can be seen in Table 8

Table 7 - Absolute values of poisonous plants and related conditions diagnosed in livestock raised in northeastern Amazon region between 1997 and 2017.

Condition/Animal species	Cattle	Buffalo	Equine	Sheep	Caprine	Total
Poisoning by *Ipomoea asarifolia*	5	2	-	1	1	9
Photossensibilization by *Brachiaria* sp.	3	-	5	8	-	16
Poisoning by *Palicourea juruana*	1	-	-	-	-	1
Colic caused by *Panicum maximum* ingestion	-	-	4	-	-	4
Traumatic lesions by *Mimosa sp.*	-	-	4	-	-	4
Poisoning by *Ipomoea carnea* sub. *fistulosa*	-	-	-	-	6	6
Total	9	2	13	9	7	40

Source: Authors.

Table 8 - Histologic findings of poisonous plants and related conditions in livestock raised in northeastern Amazon region between 1997 and 2017.

Condition	Tissue	Histologic findings
Poisoning by *Ipomoea asarifolia*	Central Nervous System	Lack of histology findings
Photossensibilization by *Brachiaria* sp.	Liver	Hepatocytes enlarged and presenting intracytoplasmic vacuoles. Accumulation of large macrophages with foamy cytoplasm (foam cells).
Poisoning by *Palicourea juruana*	Kidney	Renal tubular epithelial cells exhibiting a large intracytoplasmic vacuole that displaces a pyknotic nuclei
Colic caused by *Panicum maximum* ingestion	Intestine	Mucosa and submucosa severe infiltrated by eosinophils. The lesions are prominent in the large intestine.
Traumatic lesions by *Mimosa sp.*	Skin	Ulcerative pyogranulomatous dermatitis with fibrosis. *Mimosa* spicule can be seen in ulcerated areas
Poisoning by *Ipomoea carnea* sub. *fistulosa*	Central Nervous System	Neurons in the white and gray matter presenting large intracytoplasmic vacuoles

Source: Authors.

Our casualty of poisoning by mineral and chemical agents included copper poisoning in small ruminants and outbreaks of lead poisoning, salt poisoning, and ionophore poisoning (Table 9). However, we were not able to confirm those

diagnosis with toxicological exams, once the fresh samples were no longer available, neither the clinical files disclosed any information about those tests being performed. Therefore, this data was included solely based on the histological description.

Lead and salt poisoning presented similar histologic lesions suggestive of polioencephalomalacia (Table 10). We evaluated ten cases strong suggestive of chronic copper poisoning, eight in sheep and two in cattle. Histologic changes varied according to the clinical evolution of the disease. Most animals developed hemolytic syndrome and neuropathy; there was ischemic necrosis in the liver and kidneys, yellow pigment in the epithelial cells of renal tubules, and scatted orange hemoglobin cylinders in the kidney cortical zone. Cases with a longer clinical onset were characterized by chronic liver failure with bile stasis and fibrosis. The latter was easily detected with the aid of Masson's Trichome stain.

In our survey, copper poisoning diagnose was reinforced by identifying copper deposits in the liver with the aid of the rubeanic acid stain. Brown granules were seen within the cytoplasm of hepatocytes in positive cases.

Table 9 - Absolute values of poisoning by mineral and chemical agents diagnosed in livestock raised in northeastern Amazon region between 1997 and 2017.

Condition/Animal species	***Cattle***	***Buffalo***	***Equine***	***Sheep***	***Caprine***	**Total**
Lead poisoning	3	3	-	-	-	3
Copper poisoning	2	-	-	8	-	10
Ionophore toxicity	1	-	-	4	-	5
Salt poisoning	3	-	-	-	-	3
Total	9	3	-	12	-	24

Source: Authors.

Table 10 - Histologic findings of poisoning by mineral and chemical agents in livestock raised in northeastern Amazon region between 1997 and 2017.

Condition	**Tissue**	***Histologic findings***
Lead poisoning	Central Nervous System	Laminar cortical necrosis of neurons; enlarged astrocytes; vacuolization and patchy regions in the parenchyma (polioencephalomalacia). The diagnosis is based on neurological clinical signs, history of lead environmental contamination, and mean levels of lead in the liver and kidneys
Copper poisoning	Liver and kidney	Acute copper poisoning: Liver. Centrolobular ischemic necrosis. Hepatocytes multifocally filled with intracytoplasmic vacuoles (fatty degeneration) Kidney. Cortical tubular ischemic necrosis. Epithelial tubular cells filled with intracytoplasmic yellow gold pigment (bile). Orange hemoglobin cylinders dispersed randomly in the parenchyma. Chronic copper poisoning Liver. Accumulation of golden yellow pigment (cholestasis). Hepatocytes replaced with fibrous tissue. Copper deposits can be seen in hepatocytes with the aid of the Rubeanic Acid Stain
Ionophore toxicity	Skeletal muscle	Skeletal fibers with high eosinophilia, lack of striation and loss or architecture (floccular necrosis). Necrosis is readily seen with the aid of Masson's Trichome stain.
Salt poisoning	Central Nervous System	Laminar cortical necrosis of neurons; enlarged astrocytes; vacuolization and patchy regions in the parenchyma (polioencephalomalacia). The diagnosis is based on neurological clinical signs, and mean levels of sodium chloride in the brain

Source: Authors.

Eighty and six cases with undetermined etiology or diseases underrepresented to compose a separate entry were grouped into the miscellaneous category (Table 11). We highlight the four cases of polioencephalomalacia, the nine cases of leukoderma, and the 69 inflammatory cases.

The inflammatory changes comprised mild or unspecific acute and chronic inflammatory infiltrates affecting single or multiple organs that failed to stain in the routine bacterial and fungal histology techniques and otherwise did not direct the pathologists to any specific etiology.

Microscopic evaluation of polioencephalomalacia is described in Table 12. Those cases were negative for rabies and herpesvirus infections on immunohistochemistry and polymerase chain reaction, respectively, and could not be clinically associated with lead poisoning, salt poisoning, or thiamin deficiency.

The striking microscopic feature of leucoderma was the loss of skin pigmentation. Large patches of unpigmented epithelium made a hard contrast with the normal epidermis. The changes were readily visible with the aid of the methylene blue stain.

Table 11 - Absolute values of miscellaneous diseases in livestock raised in northeastern Amazon region between 1997 and 2017.

Disease/Animal species	***Cattle***	***Buffalo***	***Equine***	***Sheep***	***Caprine***	**Total**
Cryptosporidiosis	1	-	-	-	-	1
Eimeriosis	1	-	-	-	-	1
Polioencephalomalacia	1	1	-	-	2	4
Leukoderma	-	8	1	-	-	9
Snake bite	-	-	1	-	-	1
Urticaria	-	-	1	-	-	1
Inflammatory changes	35	10	7	14	3	69
Total	38	19	10	14	5	86

Source: Authors.

Table 12 - Histologic findings of miscellaneous diseases in livestock raised in northeastern Amazon region between 1997 and 2017.

Disease	***Tissue***	***Histologic findings***
Cryptosporidiosis	Small intestine	Small, round, and basophilic structures can be seen bordering the villi
Eimeriosis	Large intestine	Mesenteric edema, loss of mucosal architecture. Large oocysts can be seen in the mucosa
Polioencephalomalacia	Central Nervous System	Laminar cortical necrosis of neurons; enlarged astrocytes; vacuolization and patchy regions in the parenchyma
Leukoderma	Skin	Extensive areas in the epidermis in which there's abrupt melanin dyspigmentation. The lesion is readily seen with the aid of the methylene blue stain
Bothropic snake bite	Skeletal muscle	Hemorrhagic floccular necrosis and edema. Lymphoplasmacytic inflammation between the muscular fibers
Urticaria	Skin	Mild mixed superficial mononuclear perivascular dermatitis rich in macrophages, lymphocytes and plasm cells

Source: Authors.

4. Discussion

Most diseases retrieved from our survey were infectious. Infectious diseases are an important cause of death in Amazonian herds and pose an emerging concern with social and economic implications. Raising cattle and buffaloes for meat

and dairy production is the main source of income for a significant part of the Amazonian population. The spread of bacteria and viruses treats this livelihood and contributes to economic losses for local farmers and the agricultural sector. Nonetheless, common infectious diseases are zoonotic and a risk to human health (Andrade et al., 2016).

The lack of effective surveillance and control measures is the main factor for the high prevalence of infectious diseases in the Brazilian Amazon Region. The vast nature of the Amazon rainforest makes it challenging to comprehensive monitoring the herds and allow pathogens to circulate undetected within the livestock population. The unawareness of local farmers, low vaccination coverage, introduction of news animals into the herds with no respect to quarantine time, and limited access to veterinary services in remote areas hinder effective campaigns for disease control (Tauil, 2009)

The interaction between domestic animals and wildlife reservoirs is also problematic. The Brazilian Amazon region is home to various animal species, including vampire bats, boars, deer, and capybaras, potential carriers of viruses and bacteria. The extensive livestock raising practiced in Amazonia facilitates the contact of the herds with the wildlife and predisposes the infection through shared grazing habits and water contamination (Aguiar et al., 2007; Tauil, 2009).

Nonetheless, Amazon's diverse ecosystems, including rainforests and rivers, provide ideal breeding grounds for mosquitoes and bugs. The Amazonian land remains flooded for a good part of the year, creating ideal humid climate conditions for the spread of vector borne diseases, such as equine infectious anemia and pythiosis (Cook et al., 2013; Freitas et al., 2015).

Tropical soils are notoriously thin and poor in nutrients. The high temperatures and heavy rains throughout the year quickly wash away the organic matter, making the soils sandy and clayish. Only 5% of all soils in the Amazonian basin are naturally fertile. Phosphorus is the scantiest element in both concentration and availability, and an average of 30% of soils are poor in copper, zinc, and molybdenum (Luizão, 2007).

Once livestock production is practiced extensively, farm animals cannot meet minimum nutritional requirements, only feeding on the pasture. Previous studies have shown that copper, cobalt, and phosphorus deficiencies are the most common in the Amazonian herds (Tokarnia et al., 2012), the latter being a significant concern for bubaline raised in Marajó Island, regardless of age and aptitude (Oliveira, 2011).

We found histologic changes suggestive of mineral imbalances in 74 patients. Although dosage of minerals in tissue and blood samples are necessary to confirm mineral deficiencies in livestock, microscopical changes associated with certain mineral imbalances are well documented (Tokarnia et al., 2012). Therefore, the suggestive diagnose of mineral imbalances in this survey are helpful to guide the clinician but should not be used as a stand-alone criterion for definitive diagnosis.

On the other hand, although low blood levels of calcium, selenium, zinc, magnesium, and manganese have been previously described in livestock raised in the State of Pará (Bomjardim et al. 2015), we failed to identify histologic findings to support a clinical imbalance of these elements.

Poisonous plants are a significant cause of death in the Amazonian herds, only seconding hunger and other nutritional issues. Due to geographical distribution, plants that affect heart function and cause sudden death are responsible for most outbreaks in adult cattle. *Palicourea marcgravii* is extensively found throughout the pastures in the dry land, while *Tanaecium bilabiatum* (*Arrabidea*) *bilabiata* and *Friderichia* (*Arrabidea*) *japurensis* are abundant in flooded areas (Tokarnia, Döbereiner, Silva, 2007).

Cases of sudden death due to ingestion of *Palicourea* and other cardiac plants were minimal in our study. We hypothesize that veterinaries in Amazonia diagnose poisonous plant mortalities based on the history of sudden death and identification of the plant in the pasture without the aid of laboratory tests. Although a presumptive diagnosis can be built on clinical and epidemiologic features, gross and histologic examinations are essential to rule out zoonotic conditions such as rabies and botulism (Tokarnia et al., 2007).

Species of *Ipomea* sp. have limited geographical distribution compared to plants that cause sudden death, and poisoning occurs sporadically as outbreaks, similar to the results retrieved from the survey. A tremorgenic syndrome due to ingestion of *Ipomoea asarifolia* was described in bovine, buffaloes, and small ruminants in Pará and Tocantins (Tortelli et al., 2008; Costa et al., 2011; Barbosa et al., 2012;) and poisoning by *Ipomoea carnea* subs. *fistulosa* was reported in caprine from Marajó Island (Oliveira et al., 2009)

Four horses in our study died of colic after feeding in a pasture of Guinea Grass. Severe eosinophilic enteritis suggested poisoning by *Panicum maximum*. Due to their high productivity, good nutritional quality, and easy adaptation, extensive areas in the Amazon Forest are used for foraging plant species such as *Brachiaria* (*Urochola*) sp. and *Panicum* sp. It is still unclear why varieties of *Panicum* may lead to toxic disease in horses; however, a few hypotheses were postulated. Likely, overload by highly fermentable carbohydrates could be linked to the condition in the Amazon area. During the regrowth phase in the rainy season, non-structural carbohydrates are synthesized by the grass and readily fermented by the equine gut microbiota, causing colic (Cerqueira et al., 2009; França et al., 2021).

Hepatogenous photosensitization is a known issue in livestock grazing *Brachiaria (Urochloa)* in the Amazon basin. The grass is rich in steroidal lithogenic saponins, a chemical compound metabolized by the liver that induces deposits of biliary crystals and the onset of secondary photosensitization. The amount of saponins in *Brachiaria (Urochloa)* varies according to environmental stress, plant age, and developmental phase, so poisoning outbreaks are sporadic. The disease may occur any time of the year, and younger animals are more susceptible than adults. Livestock first introduced in pastures of *Brachiaria (Urochloa)* are more likely to develop photosensitization, so an adaptive period is recommended, especially for animals under two years of age (Albernaz et al., 2010).

In this study, copper deposits were seen in the liver of eight sheep with the aid of the Rubeanic Acid stain. Sudden (acute) copper poisoning is rare, and the disease usually results from long-term exposure. When the animal is under stress, the copper accumulated in the liver can be released into the bloodstream, leading to hemolytic syndrome, hepatic failure, and death (Miguel et al., 2013).

Feeding sheep with a commercial bovine mineral mix is the main predisposing factor for copper poisoning. All copper ingested by sheep is absorbed by the organism, regardless of the amount in the diet, unlikely other mineral substances. Following ingestion, copper is gradually stored in the liver, and toxic levels can be noticed months or years after the first intake (Lemos et al., 2007). Considering the Amazon basin has soils notorious poor in nutrients and commercial mineral mixes commonly aid the diet in the herds, small ruminants in the area are susceptible to copper poisoning. Therefore, farmers should be careful with the food given to ovine and caprine once their dietary requirements differ completely from cattle and bubaline (Tokarnia et al., 2012).

Veterinaries have noticed cases of a lymphoproliferative condition in the northeastern Brazilian Amazon Region since 2006. Affected bubaline developed visceral and subcutaneous nodules and presented clinical signs of lymphadenopathy and progressive weight loss. Histologic and immunohistochemical evaluation of the nodules showed a malignant, infiltrative, and invasive lymphoid neoplasia that exhibited immunoreactivity with the CD79 antibody, ergo classified as a B-cell lymphoma. On immunohistochemical and molecular evaluation, the tissue samples were negative for the Bovine Leukemia Virus, the bovine herpesvirus 6, and the bovine immunodeficiency virus. The authors named this entity as bubaline multicentric lymphoma and described it as a chronic disease similar to bovine enzootic leukosis. Viral involvement is suspected but yet not proven (Oliveira et al., 2016).

Nine buffaloes presented areas of skin depigmentation compatible with leukoderma. Leucoderma is a rare skin condition of horses, cattle, pigs, and buffaloes (Sako, 1983). It is a multifactorial disease whose exact etiology remains

unknown. Previous studies suggested that genetic, immunologic, and immunological features could be involved in the pathogeny of leukoderma (Alikhan et al., 2011). Ramaiah et al. (1989) hypothesized that a decrease in the melanin growth factor would lead to skin depigmentation, while Schallreuter et al. (1991) believed that leukoderma was a disease of keratinocytes. Muhammad et al. (1998) associated diet abnormalities (molybdenum poisoning and copper deficiency) with skin depigmentation in farm animals and suggested that large doses of copper sulfate would reverse the condition.

Recently, scientists obtained excellent results treatment leukoderma in buffaloes feeding them a mineral mix *ad libitum* containing 406mg/kg of copper sulfate for 90 days. The single buffalo that failed to respond to this treatment was given 5mg/kg of intramuscular dexamethasone for 30 days, along with the copper supplementation. It resulted in the complete cure of the illness (Barbosa et al., 2023). In our study, we failed to associate possible etiologies and predisposing factors in the livestock diagnosed with leukoderma, and the clinical files did not contain information on the treatment of choice in those cases.

5. Conclusion

Bovine and bubaline were the species that demanded more assistance from histopathological evaluation. The data provided by the archives of Anatomical Pathology Sector of the Federal Rural University of Rio de Janeiro showed that infectious diseases, mineral imbalances, neoplasia, poisonous plants, and related conditions prevailed; while cobalt deficiency, equine infectious anemia, copper deficiency, rabies, and brucellosis were the most common entities affecting livestock in northeastern Brazilian Amazon Region. Moreover, histochemical, immunohistochemical, and molecular techniques can be useful in clarifying obscure diagnosis and aid retrospective studies.

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