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Clinical and anatomopathological findings of lipid-related lesions in wild and pet birds from the State of Paraíba, Northeastern Brazil[1]

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ABSTRACT.- Oliveira-Filho H.S., Duarte J.L.C., Paranhos G.F., Oliveira R.L., Farias R.C. & Leal de Araújo J. 2023. **Clinical and anatomopathological findings of lipid-related lesions in wild and pet birds from the State of Paraíba, Northeastern Brazil.** *Pesquisa Veterinária Brasileira 43:e07296, 2023*. Departamento de Ciências Veterinárias, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Campus II, Rodovia 12, Areia, PB 58397-000, Brazil. E-mail: lealjeann@gmail.com

Diseases related to lipid metabolism disorders are reported in several orders of birds, especially in psittacines, and include obesity, atherosclerosis, hepatic lipidosis, egg yolk coelomitis, lipomas, liposarcomas, xanthomas and xanthogranulomas. This study describes epidemiological, clinical and anatomopathological aspects of 28 cases involving lipid-related lesions in wild and pet birds from Northeastern Brazil. The cases were selected from 313 avian patients referred to the UFPB Veterinary Hospital from 2018 to 2022. Lipid-related tumors were the most frequent lesions, followed by obesity, hepatic steatosis, egg yolk coelomitis, atherosclerosis and lipemia. The Psittaciformes order was the most affected, and an erroneous diet (excess consumption of sunflower seeds, bread, crackers, rice, etc.) was identified as the main risk factor for the development of these lesions, which reinforces the need to warn bird owners and caretakers about the importance of adequate nutrition. Forty-nine percent of the reported tumors were in the pericloacal region, which makes lipomas and xanthomas an important differential diagnosis for nodules in this location of birds, especially psittacids.

INDEX TERMS: Atherosclerosis, avian pathology, lipoma, xanthomas, lipid, birds, Brazil.

RESUMO. - [Achados clínicos e anatomopatológicos de lesões relacionadas à desordens lipídicas em aves silvestres e pet do Estado da Paraíba, Nordeste do Brasil.] Doenças relacionadas à desordens no metabolismo dos lipídeos são reportadas em diversas ordens de aves, especialmente em piscítacídeos e incluem obesidade, aterosclerose, lipidose hepática, celomite por gema de ovo, lipomas, lipossarcomas, xantomas e xantogranulomas. Este estudo descreve aspectos epidemiológicos, clínicos e anatomopatológicos de 28 casos envolvendo lesões relacionadas a lipídeos em aves silvestres e pet no Nordeste Brasileiro. Os casos foram selecionados de um total de 313 aves atendidas entre 2018 e 2022 no Hospital Veterinário da UFPB. Tumores relacionados a lipídeos foram as lesões mais relatadas, seguido por obesidade, esteatose hepática, celomite por gema de ovo, aterosclerose e lipemia. A ordem Piscitaciforme foi a mais acometida e dieta errônea (Excesso de sementes de girassol, pão, bolacha, arroz, etc.) foi identificado como o principal fator de risco para o desenvolvimento das lesões, o que reforça a necessidade de advertir os tutores quanto a importância da alimentação adequada. Quarenta e nove por cento dos tumores relatados estavam na região pericloacal, o que torna lipomas e xantomas um diagnóstico diferencial importante para nódulos nessa localização em aves, principalmente psitacídeos.

TERMOS DE INDEXAÇÃO: Aterosclerose, lipoma, patologia aviária, xantomas, lipídeos, aves, Brasil.

INTRODUCTION

In pet birds, lipid-related disorders include diseases such as obesity, atherosclerosis, hepatic lipidosis, fatty tumors (lipoma, liposarcoma), egg yolk coelomitis, xanthomas and xanthogranulomas (Lujan-Vega et al. 2021, Donovan et al.

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2022, Wildmann et al. 2022). Although these conditions are more frequently reported in psittacine birds (Beaufrère et al. 2019), they can also be observed in several other orders of birds, such as Pelicaniformes, Accipitriformes, Falconiformes, Strigiformes, and Passeriformes (Sanches 2008, Lujan-Vega et al. 2021, Wildmann et al. 2022).

The occurrence of diseases related to lipid metabolism may be associated with nutritional factors, especially cholesterol-rich diets, and varies depending on the geographic location, number of animals used in the study, age, sex, species and inclusion criteria (Castro et al. 2016, Beaufrère et al. 2019, Lujan-Vega et al. 2021). To date, there are no peer-reviewed studies that characterize these diseases in birds in Northeastern Brazil. This study describes the clinical and pathological aspects of lipid-related lesions found in wild and companion birds from 2018 to 2022 in the state of Paraíba, Northeastern Brazil.

MATERIALS AND METHODS

The study was approved by the Ethics Committee on Animal Use of the "Universidade Federal da Paraíba" (CEUA-UFPB 4998050520) and it was conducted under license SISBIO ("Sistema de Autorização e Informação em Biodiversidade" - Authorization and Information System in Biodiversity) no. 83866/1.

Clinical and histopathology findings (necropsies and biopsies) of cases involving lesions related to lipid disorders were collected from the records of the Veterinary Hospital (HV) at the UFPB, Areia, Paraíba, Northeastern Brazil. All birds included were referred to the Hospital from 2018 to 2022.

All bird records were reviewed, and cases were selected based on descriptive terms related to neoplasms of adipose tissue, degenerative lesions associated with lipid metabolism, xanthomatous or xanthogranulomatous lesions, atherosclerosis, lipemia (lipemic plasma, serum or blood) and egg yolk coelomitis. Birds that presented simultaneously more than one lesion were included in the "multiple lesions" category.

Histology slides stained with hematoxylin and eosin (HE) were reviewed, and new sections were obtained when necessary. Masson's trichrome stain was used for suspected cases of atherosclerosis to identify chondroid metaplasia. The weight of the birds was compared to reference values published elsewhere (Dário et al. 2002, Vianna 2010, Grespan & Raso 2014, Bagh et al. 2016, Rodrigues et al. 2019, Baldotto et al. 2021).

RESULTS

Of the 313 birds referred to the HV-UFPB from 2018 to 2022, twenty-eight cases (8.9%) met the inclusion criteria. The selected cases included 14 psittacines (50%), eight passerines (28.6%), four gallinaceous birds (14.3%), one water bird (3.6%) and one raptor (3.6%).

The birds included were diagnosticated with lipomas (32.2%), xanthomas (10.7%), hepatic steatosis (10.7%), egg yolk coelomitis (7.1%) and obesity (17.9%). Another twenty-one percent (21.4%) of birds had multiple lesions, and 83% of these cases were associated with obesity. Considering the cases of multiple lesions, obesity was found in 35% of the birds included in this category. One case of atherosclerosis and one case of lipemia were also diagnosed among the birds with comorbidities. Figure 1 describes all lipid-related lesions diagnosticated in this study according to location, species and percentage of occurrence.

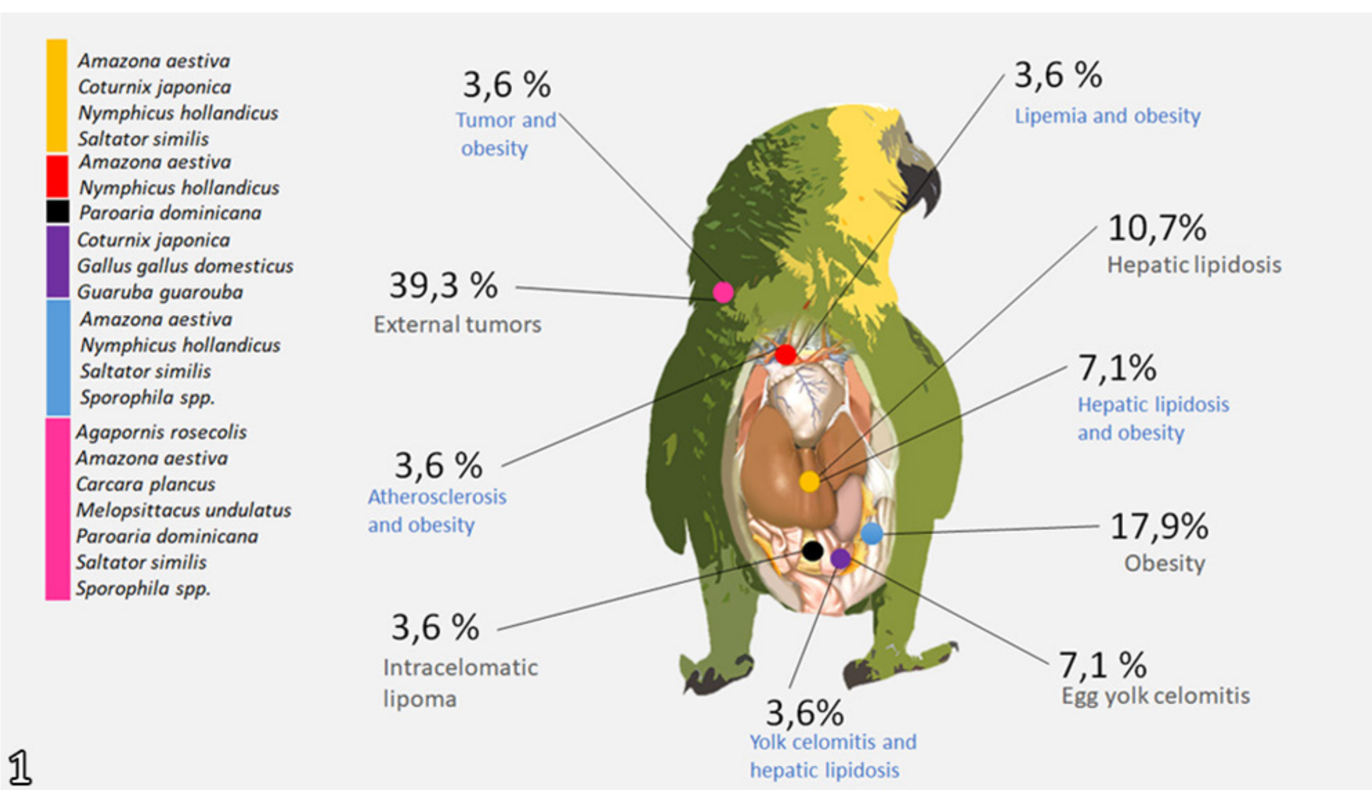

Fig.1. Lipid-related lesions in wild and pet birds from the State of Paraíba, Northeastern Brazil, according to anatomic sites, affected species and percentage of occurrence.

Thirty-nine percent (39.2%) of the birds were female, and 17.8% were male. In other 42% of the cases, the owners could not determine the sex of the birds. Thirty-nine percent (39.9%) of the birds received an inadequate diet, and 39.3% of the records did not contain any information regarding food intake. Among Psittaciformes, order most affected, all birds that had available dietary information received inadequate food (excess consumption of sunflower seeds, bread, crackers, pasta, coffee) and new feeding protocols were recommended to the owners, according to the species and individual characteristics of the birds.

The most frequent clinical signs were sudden death (50%), followed by tumor-like lesions (42%), obesity (14.2%), pododermatitis (10.7%), apathy (3.5%) and lipemic serum (3.5%). Sixty-two percent of the animals with weight information available were considered overweight compared to reference values for the species.

Forty-two percent (42.8%) of the birds (12/28) had subcutaneous tumors classified as lipomas (9/12) or xanthomas (3/12) through cytology and histopathology, and sixty-three percent (63.63%) had internal lesions. Fat accumulation inside the coelomic cavity (Fig.2 and 3), characterizing obesity, accounted for 47.6% of internal lesions, followed by hepatic steatosis (28.6%), egg yolk coelomitis (14.3%), atherosclerosis (4.8%) or intracoelomic lipoma (4.8%). These lesions are listed according to the affected species and number of cases in Table 1.

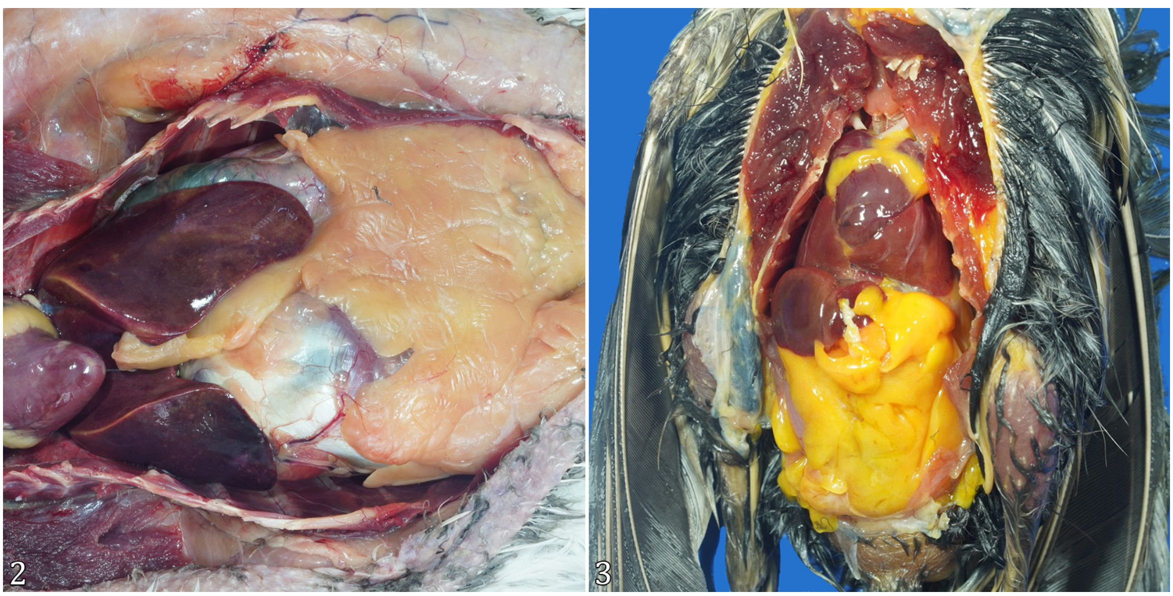

Fig. 2-3. Gross appearance of obesity in birds during necropsy. (**2**) Extensive areas of adipose tissue deposition in the coelomic cavity of an obese swan goose (*Anser cygnoides*). (**3**) Large amount of adipose tissue deposition inside the coelomic cavity of an obese green-winged-saltator (*Saltator similis*).

Table 1. Epidemiological data of wild and pet birds diagnosed with lipid-related lesions from January 2018 to December 2022

Specie	N	Sex	Age (years)	Weight (average)	Lipid-related lesions
Lovebird (*Agapornis roseicollis*)	3	1 male, 1 female, 1 unknown	8.0-9.0	62g	Lipoma, xanthoma
Turquoise-fronted amazon (*Amazona aestiva*)	5	1 male, 1 female, 3 unknown	30.0	676g	Lipoma, obesity, hepatic steatosis, atherosclerosis
Cockatiels (*Nymphicus hollandicus*)	4	1 male, 1 female, 2 unknown	2.0-6.0	93g	Lipoma, xanthoma obesity, hepatic steatosis, lipemia
Budgerigars (*Melopsittacus undulatus*)	1	Female	10.0	65g	Lipoma
Golden parakeet (*Guaruba guarouba*)	1	Female	8.0	NI	Egg yolk coelomitis
Green-winged saltator (*Saltator similis*)	4	1 male, 3 unknown	3.0	60g	Lipoma, obesity, hepatic steatosis
Seedeater (*Sporophila* spp.)	3	1 female, 2 unknown	NI	17g	Lipoma, obesity
Red-cowled cardinal (*Paroaria dominicana)*	1	Male	NI	60g	Intracelomic Lipoma
Crested carcara (*Caracara plancus*)	1	Unknown	NI	890g	Xanthoma
Swan Goose (*Anser cygnoides)*	1	Female	2.0	3000g	Obesity
Japanese quail (*Coturnix japonica*)	3	Female	0.3	154g	Hepatic steatosis, egg yolk coelomitis
Domestic chicken (*Gallus gallus domesticus*)	1	Female	1.0	NI	Egg yolk coelomitis

N = Number of animals.

Lipomas were described as soft, skin-covered subcutaneous solitary or multiple masses that were mobile and yellowish on the cut surface (Fig.4 and 5), while xanthomas were soft to firm, pendulous masses, covered by featherless skin (Fig.6). One of the xanthomas, located in the cervical region of a lovebird (*Agapornis roseicollis*), had a cystic cavitation with a large amount of free green fluid and a firm core at its center (Fig.7). Another xanthoma, located at the pericloacal region of a cockatiel (*Nymphicus hollandicus*), had multifocal ulcerated and hemorrhagic areas, probably due to contact with the cage surface. Microscopically, lipomas were characterized as non-encapsulated and well-circumscribed neoplasms, composed of a monomorphic population of mature adipocytes, separated by thin fibrovascular stroma with rare or absent mitotic figures (Fig.8) differing from xanthomas, which were characterized by lipid-laden macrophages, multinucleated giant cells, and numerous linear, clear, acicular-shaped structures (cholesterol clefts) with cell debris and moderate epidermal hyperplasia (Fig.9).

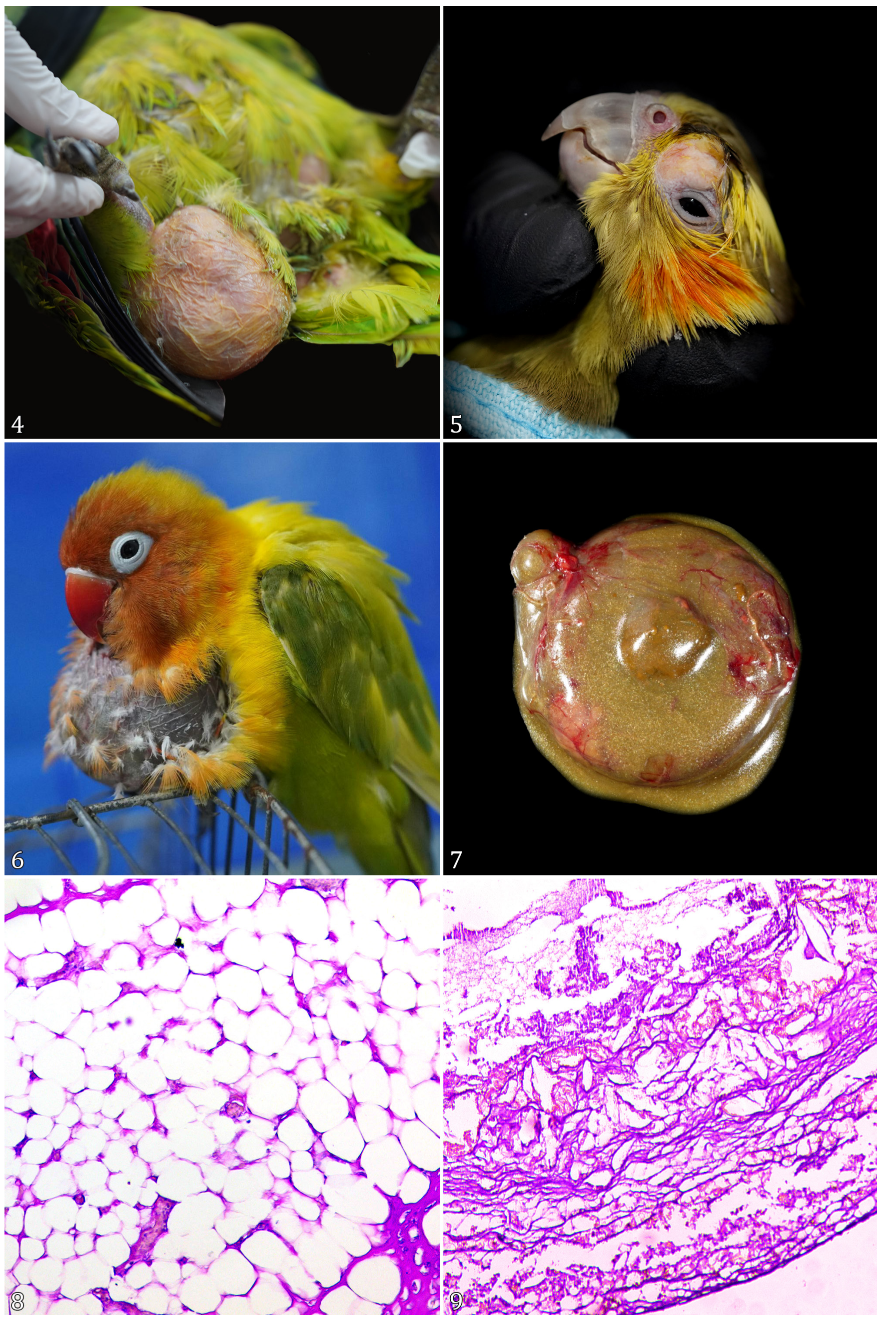

Fig.4-9. Gross and microscopic findings of lipomas and xanthomas in pet birds. (**4**) Lipoma in the pelvic limb of a turquoise-fronted-parrot (*Amazona aestiva*). (**5**) Periocular lipoma in a cockatiel (*Nymphicus hollandicus*). (**6**) Xanthoma in the cervical region of a lovebird (*Agapornis roseicollis*). (**7**) Cut surface of the xanthoma from Figure 7. Note cystic cavitation with a large amount of free green fluid surrounding a firm central core. (**8**) Microscopic appearance of a lipoma in a turquoise-fronted-parrot (*A. aestiva*). HE, obj.20x. (**9**) Xanthoma in a lovebird (*A. roseicollis*). Note the large amount of acicular structures (cholesterol clefts). HE, obj.20x.

Seventy percent of the birds affected by lipomas (cutaneous or intracoelomic) were parrots (7/10), and 30% were passerines (3/10). Xanthomas were diagnosed in two psittacines and one raptor. The tumors were found mainly in the pericloacal region (49%) but also on the face (16.6%), cervical region (16.6%), pelvic limbs (8.3%) and wings (8.3%). Cutaneous lipomas affected the cervical region (1/9), head (2/9), pelvic limbs (1/9) and pericloacal region (5/9), while xanthomas affected wings (1/3), cervical (1/3) and pericloacal regions (1/3). Figure 10 illustrates the location of cutaneous lipomas and xanthomas according to the location, species and percentage of occurrence.

The surgical procedure was performed in all cases of xanthoma and one case of lipoma involving the pelvic limb of a turquoise-fronted parrot (*Amazona aestiva*). In these four cases, the birds recovered completely a few days after the surgery. Except for the only case of lipoma submitted to surgery and one case of lipoma diagnosed during necropsy, all other birds diagnosed with lipomas show a significant reduction of masses or cure only with dietary changes.

We found a case of atherosclerosis in an obese turquoise-fronted amazon (*A. aestiva*) of unknown age and sex. At necropsy, pulmonary and aorta arteries were diffusely yellowish and thickened (Fig.11), with a reduced lumen. The heart showed pale multifocal areas in the myocardium (Fig.12). Microscopically, the affected vessels had large areas of lipid deposition, forming a lipid core, associated with disorganization and thickening of the arterial wall with disarrangement of elastin fibers (Fig.13 and 14). Masson's special trichomic staining showed chondroid metaplasia and allowed better visualization of atherosclerotic plaques distributed in layers on the vessel wall (Fig.15).

Hepatic steatosis was identified in six animals of three different orders: three Japanese quails (*Coturnix japonica*) referred for necropsy with a history of sudden death, a six-year-old cockatiel (*N. hollandicus*) with a history of apathy, a turquoise-fronted amazon (*A. aestiva*) and a green-winged saltator *(Saltator similis*), both presenting obesity. A yellowish liver parenchyma was observed in all cases, and liver fragments floated when collected in a 10% formalin solution. The hepatocytes showed moderate to marked cytoplasmic vacuolation (vacuolar degeneration, lipid type) with peripheralized nuclei (Fig.16).

A Japanese *quail (C. japonica*) that also had hepatic lipidosis, a chicken (*Gallus gallus domesticus*) and a golden parakeet (*Guaruba guarouba*) with a history of sudden death was diagnosed with egg yolk coelomitis (yolk peritonitis) during necropsy. In those cases, there was a rupture of ovarian follicles with a large deposition of fibrin and yolk material in the peritoneal cavity. There was adhesion of the intestines, liver and follicles and congestion of oviduct serosal blood vessels, and the diagnosis was made only by gross lesions.

One case of lipemia was diagnosed in an adult cockatiel, referred to the HV-UFPB, with beak overgrowth and obesity. The diagnosis was based on a hematological examination, which found lipemic serum in a blood sample collected via the jugular vein.

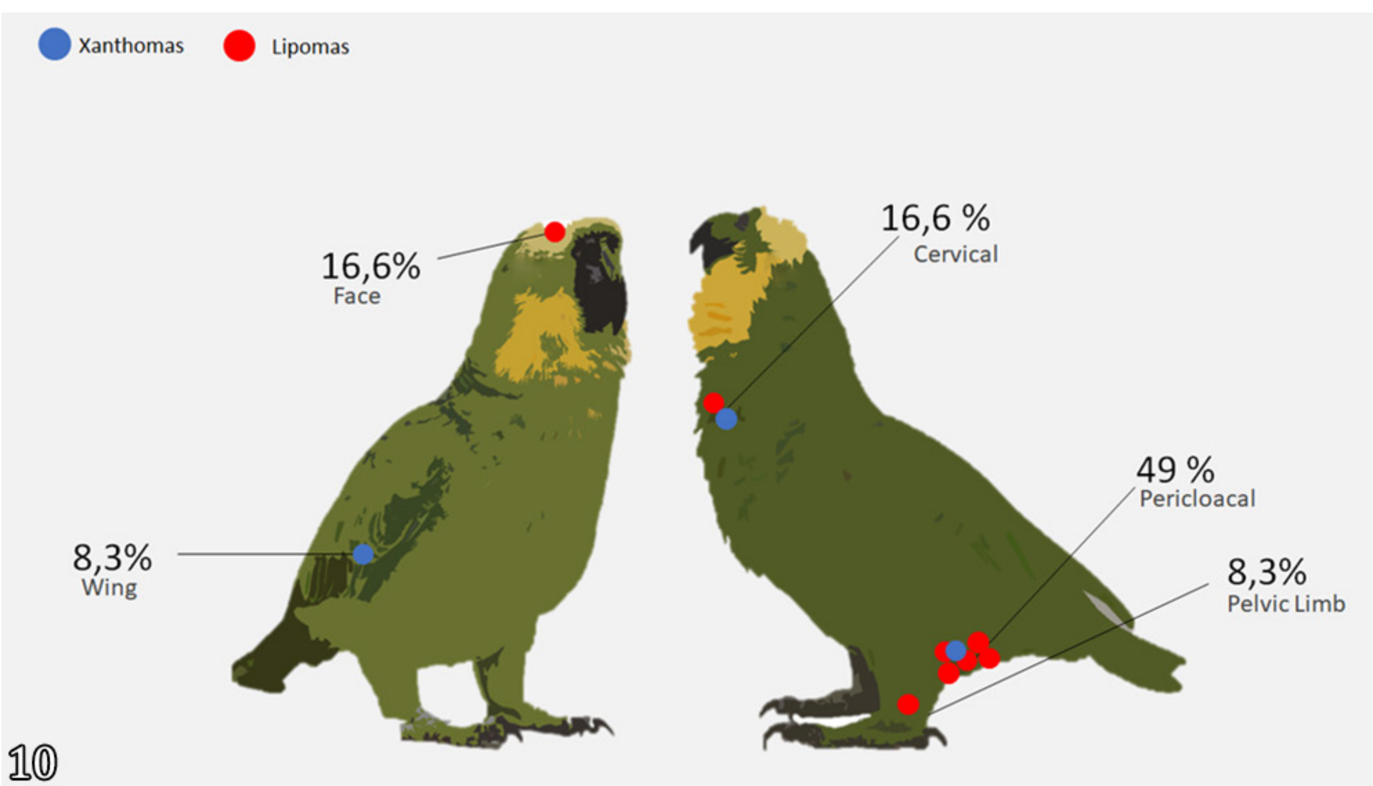

Fig.10. Location of tumors associated with lipid disorders diagnosed in wild and pet birds at the "Universidade Federal da Paraíba" (UFPB) from 2018 to 2022.

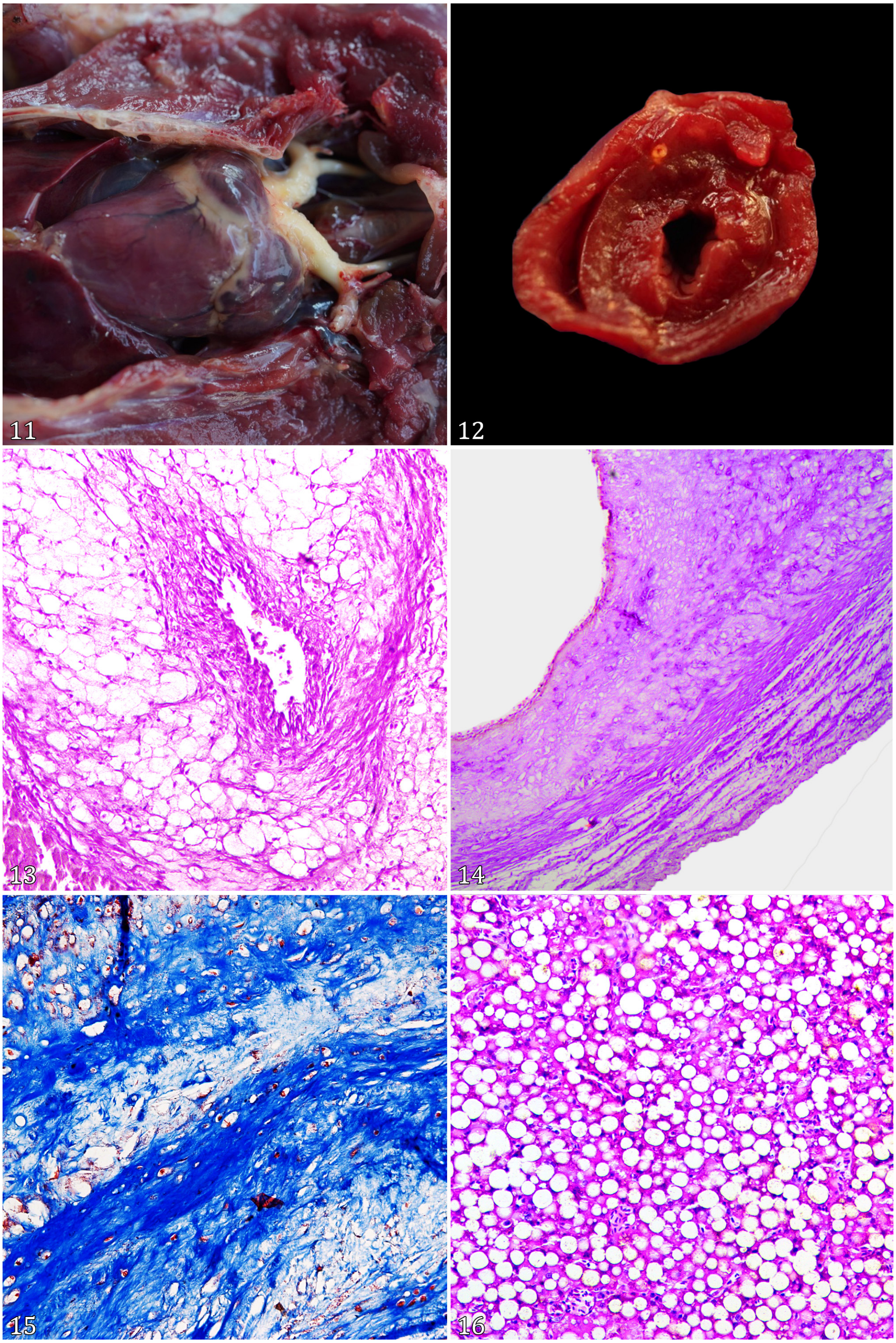

Fig.11-16. Gross and microscopic findings of lipid-related lesions in wild and pet birds from Northeastern Brazil. (**11**) Diffuse thickening and yellow discoloration of a turquoise-fronted-parrot's pulmonary arteries and aorta (*Amazona aestiva*). (**12**) Cross-section of the heart from Figure 11 showing a yellowish focal area corresponding to atherosclerotic lesions in a smaller artery. (**13**) Atherosclerosis affecting the great vessels of a parrot's heart. Lipid deposition in the wall of a pulmonary artery of a turquoise-fronted parrot (*A. aestiva*). Note the deposition of lipid content on the wall of a pulmonary artery HE, obj.40x. (**14**) Aorta artery of a turquoise-fronted parrot (*A. aestiva*) shows wall thickening due to deposition of a lipid core. HE, obj.20x. (**15**) Aorta artery of a turquoise-fronted parrot (*A. aestiva*). Derangement of elastin fibers and layers of atherosclerotic plaques deposited on the vessel wall. Masson's trichrome, obj.40x. (**16**) Diffuse lipid-type vacuolation of hepatocytes in a parrot (*Amazona* spp.). HE, obj.10x.

DISCUSSION

This study evaluated lesions related to lipid metabolism disorders in five orders of wild and pet birds. Psittacines were the most affected order, which has been corroborated by other studies that attribute this result to their popularity as pet birds, life expectancy and natural predisposition (Castro et al. 2016, Beaufrère et al. 2019). In the current study, inadequate diet seems to play a major role in the development of lipid-related lesions, which is in accordance with other studies (Crespo & Shivaprasad 2013) and, although the number of affected females was twice as high as that of males, more studies are needed in order to understand whether or not there is a sex predisposition.

Tumors related to lipid disorders were the most frequent pathology found in that study, and lipomas were the most common of these tumors. Lipomas are frequently observed in obese birds, especially parrots, budgerigars and macaws (Robat et al. 2017) and represented 40% of all neoplasms diagnosed in birds from 2018 to 2022 at the Pathology Laboratory of the UFPB, almost twice as high as that reported by other authors (Castro et al. 2016, Beaufrère et al. 2019).

Lipomas are well-circumscribed soft masses, microscopic, characterized by a population of well-differentiated adipocytes. Evidence of inflammation or necrosis is very common due to the contact of masses with cage surfaces (Robat et al. 2017). However, in this study, these findings were not observed in lipomas. Cytology is useful for diagnosing lipomas and was performed whenever possible, but contents often dissolved during the staining process.

Surgical excision can be a therapeutic option in extreme cases, but most lipomas regress once the overweight problem is corrected (Robat et al. 2017). In this study, except for one case of lipoma involving a turquoise-fronted parrot submitted to surgical treatment and one case diagnosed at necropsy, all animals showed significant improvement or healing only with changes in diet. In 80% of the cases in this study, the birds affected by lipomas were overweight.

Xanthomas, the second type of lipid-related tumor found in this study, are non-neoplastic lesions formed by macrophages, multinucleated giant cells and cholesterol clefts and often are related to hypercholesterolemia. These tumors show little or no clinical difference with lipomas and also can be traumatized by contact with the surface of cages, which was observed in one case of this study. In this case, the tumor was located in the pericloacal region and was traumatized by the cage floor.

Some xanthomas have adipose tissue regions; in sporadic cases, xanthomas may also develop from lipomas confusing the cytological diagnostic (Grespan & Raso 2014). However, the visualization of acicular structures (cholesterol clefts), characteristic of xanthomas, is useful for diagnosing (Donovan et al. 2022).

Xanthomas in birds are related to hypercholesterolemia, obesity and traumatic lesions (Souza et al. 2009). Although the relation between xanthoma development and local tissue injury (trauma or inflammation) still is not completely understood, leakage of lipoproteins of blood due to increases of vascular permeability in situations of tissue damage has been postulated with a probable pathway to the development of this lesions in humans, especially in cases of normocholesterolemia (Bell & Shreenath 2022).

In this study, we report an atypical presentation of xanthoma with cystic cavitation filled with greenish fluid. We believe that, in this case, the xanthoma was caused by trauma with subsequent hemorrhage due to the formation of hematoidin crystals, observed through light microscopy. Hematoidin is a pigment derived from hemoglobin and formed under conditions of low oxygen concentration, as occurs in hematomas (Martinez-Giron et al. 2020). The greenish fluid observed in this case is probably related to red blood cell degradation because, in hemorrhagic processes, porphyrin released from free hemoglobin is converted into biliverdin, a greenish pigment (Gáll et al. 2019).

We described a case of atherosclerosis in an obese turquoise-fronted Amazon (*Amazona aestiva*). In birds and mammals, This degenerative and inflammatory vascular disease is characterized by the deposition of lipids, inflammatory cells, calcium and collagen associated with fibroproliferation. This results in disorganization of the arterial wall, lumen stenosis, cardiovascular dysfunction and potential thromboembolic complications (Beaufrère et al. 2013).

The histopathological lesions observed in this case were classified as atheromas (Type IV) and fibroatheromas (Type V) following the system developed by Beaufrère et al. (2011). Atheromas are lesions identified by large areas of lipid deposition, forming a lipid core, associated with disorganization of the arterial wall architecture due to atheromatous plaques in the lumen. At the same time, fibroatheromas comprise an increased presence of fibromuscular tissue forming a fibrous cap over the lipid core, calcification, significant luminal stenosis and endothelial surface defects.

Histochemical and immunohistochemical methods can also be used to diagnose and classify atherosclerotic lesions in birds. (Taatjes et al. 2000, Fricke et al. 2009). In the case reported in the current study, Masson's trichrome was used to show chondroid metaplasia and highlight the atherosclerotic plaques amid collagen fibers. However, other special stains like Oil Red and Sudan Black can be useful (Tummidi et al. 2019, Zhu et al. 2019). Sudan Black is considered the most sensitive and specific stain for lipids and is used in atherosclerosis cases to highlight lipids deposition in blood vessels. However, the need for frozen sections can be a limitation in applying this technique (Arregui et al. 2020).

Although all psittacines are susceptible, birds of the genera *Amazona*, *Psittacus* and *Nymphicus* are more predisposed to the development of atherosclerosis (Beaufrère et al. 2013). Due to the lack of clinical information, it was not possible to determine which risk factors, beyond obesity, contributed to the development of atherosclerosis in this case. However, atherosclerotic lesions can be initiated by endothelial dysfunction and associated lipemia, obesity, inadequate diet, diabetes mellitus, hypertension, genetic factors and chronic infections (Beaufrère et al. 2011).

Migratory birds can store up to 60% of their body weight in fat due to cellular and enzymatic adaptations that improve lipid oxidation, making it possible to fly for more than a week without feeding (Guglielmo 2018). However, for most species, obesity is a risk factor for atherosclerosis, lipid-related tumors, lipemia, xanthomatous/xanthogranulomatous diseases and hepatic steatosis (Robat et al. 2017, Lujan-Vega et al. 2021, Donovan et al. 2022).

Hepatic lipidosis is a metabolic disorder characterized by excessive deposition of triglycerides in hepatocytes and affects mostly obese birds. Physiological hepatic lipidosis occurs as a result of natural events that increase the hepatic release of lipoproteins into the circulation, such as vitellogenesis (Middendorf et al. 2021), while the pathological form occurs due to malnutrition, liver damage or systemic diseases that cause weight loss (Rodríguez et al. 2018).

In this study, all animals that presented hepatic steatosis were sedentary due to captivity, and many received excessively caloric diets. These are the main risk factors for developing this condition. Although not observed in this study, affected birds may display greasy plumage (Wünschmann et al. 2018).

Laying birds are predisposed to hepatic steatosis because the intense production of eggs increases the concentration of estrogen in the bloodstream favoring hepatic lipogenesis (Divers & Cooper 2000). In several cases, hepatic lipidosis interferes with liver function, and the mortality rate can reach 15%. Generally, females between 10 and 14 weeks of age are more affected (Middendorf et al. 2021).

Egg yolk coelomitis is a reproductive pathology characterized by an inflammatory reaction of the peritoneum due presence of yolk material in the coelomic cavity (Reavill & Dorrestein 2018). Yolk material by itself can induce an inflammatory process in the coelomic cavity, but secondary *Escherichia coli* infection is frequently involved (Fulton 2017). This condition is most often diagnosed late when the animals are moribund or during necropsy (Gardner & Barrows 2010). In the cases we report, egg yolk coelomitis was diagnosed during necropsy, and no bacterial agents were isolated.

The major limitations of this study are related to missing clinical information, a problem also reported by other authors (Beaufrère et al. 2019). Age and sex, in particular, were the most frequently missing data, mainly because the species included showed no sexual dimorphism. To overcome this obstacle, clinicians can collect feathers for sexing. This low-cost and accessible technique would allow a better understanding of the predisposition to lipid-related lesions according to sex and species of wild and pet birds (Kroczak et al. 2021).

CONCLUSIONS

Lipid-related lesions are important findings for pet and wild birds analyzed in this study. However, further investigations are needed to understand the general picture of their occurrence in Northeastern Brazil.

The high occurrence of lipid-related lesions in psittacine birds reinforces the need to warn bird owners about the importance of an adequate diet for these species, considering that obesity is one of the main risk factors.

Additionally, lipomas should be considered a differential diagnostic for soft tumors in the pericloacal region of psittacine birds.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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