

Aspergillosis in domestic and wild birds in northeastern Brazil: 2000-2022

Page 1 a 10

[Aspergilose em aves domésticas e selvagens no Nordeste de Brasil: 2000-2022]

E.P.F. Souto^{1,2,3} , D.S. Garcia² , C.S.A.B. Santos² , J.S. Batista³ , G.D. Kommers⁴ ,
G.J.N. Galiza² , R.A. Mota¹ , A.F.M. Dantas² 

¹Postdoctoral Research Internship, National Council for Scientific and Technological Development, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil

²Postgraduate, Universidade Federal de Campina Grande, Patos, PB, Brasil

³Universidade Federal Rural do Semi-Arido, Mossoro, RN, Brasil

⁴Universidade Federal de Santa Maria, Santa Maria, RS, Brasil

ABSTRACT

The epidemiologic, clinical, pathological, immunohistochemical, and microbiological findings of aspergillosis in birds in northeastern Brazil are described in this study. From January 2000 to December 2022 the Laboratory of Animal Pathology of the Federal University of Campina Grande performed 357 necropsies on birds, 16 (4.48%) of which were diagnosed as aspergillosis. The disease affected domestic and wild birds, including quails, chickens, parrots, and a penguin. Affected animals were predominantly males, with ages ranging from 1-month-old to elderly; and reared in intensive, extensive or domiciliated systems, except for the penguin, which was free-living. The clinical course ranged from acute to chronic and clinical signs were mostly nonspecific or related to the respiratory system. Necropsy revealed lesions primarily in the respiratory tract, characterized by caseous nodules mainly in the air sacs and lungs, but there was also involvement of the liver, heart and crop. Histopathology revealed granulomatous and heterophilic inflammation associated with fungal hyphae. The diagnosis was based on epidemiologic, clinical, anatomopathological, immunohistochemical and microbiological findings. Aspergillosis affects both domestic and wild birds in the form of sporadic cases and outbreaks, causing significant economic losses in poultry farms. The disease must be included in the differential diagnosis of respiratory, digestive, and multisystemic disorders.

Keywords: avian disease, respiratory infection, hyphae, *Aspergillus* spp.

RESUMO

Descrevem-se os achados epidemiológicos, clínicos, patológicos, imuno-histoquímicos e microbiológicos da aspergilose em aves no Nordeste do Brasil. De janeiro de 2000 a dezembro de 2022 o Laboratório de Patologia Animal da Universidade Federal de Campina Grande realizou 357 necropsias em aves, das quais 16 (4,48%) foram diagnosticadas como aspergilose. A doença afetou aves domésticas e selvagens, incluindo codornas, galinhas, papagaios e um pinguim. Os animais afetados eram predominantemente machos, com idades variando de 1 mês a idosos; e criados em sistema intensivo, extensivo ou domiciliado, com exceção do pinguim que era de vida livre. O curso clínico variou de agudo a crônico e os sinais clínicos foram em sua maioria inespecíficos ou relacionados ao sistema respiratório. A necropsia revelou lesões principalmente no trato respiratório, caracterizadas por nódulos caseosos nos sacos aéreos e pulmões, mas também houve envolvimento do fígado, coração e ingluvie. A histopatologia revelou inflamação granulomatosa e heterofílica associada a hifas fúngicas. O diagnóstico foi estabelecido com base nos achados epidemiológicos, clínicos, anatomopatológicos, imuno-histoquímicos e microbiológicos. A aspergilose afeta aves domésticas e selvagens sob a forma de casos individuais e surtos, causando significativas perdas econômicas nas criações avícolas. A doença deve ser incluída no diagnóstico diferencial de distúrbios respiratórios, digestivos e multissistêmicos das aves.

Palavras-chave: doença de ave, infecção respiratória, hifas, *Aspergillus* spp.

INTRODUCTION

Aspergillosis is an infectious disease caused by fungi of the genus *Aspergillus* spp., which are saprophytic microorganisms found in soil, decaying vegetation, seeds, and grains (Arné, *et al.*, 2021). These filamentous fungi thrive as saprophytes in a wide range of environments, but also have the potential to infect living hosts including plants, insects, mammals, and birds (Seyedmousavi, *et al.*, 2015). The infection typically occurs after the inhalation of the ubiquitously available fungal spores and may range from localized to fatal disseminated conditions (Arné, *et al.*, 2021).

In fact, aspergillosis is often referred as the most common opportunistic infection of the respiratory tract of mammals and birds, causing high morbidity and mortality rates (Leishangthen *et al.*, 2015). The Aves class is particularly concerned, due to the great diversity of species susceptible, including domestic and wild animals living in captivity or in natural environments (Arné *et al.*, 2021) and to anatomical and physiological factors related to the respiratory and immunological systems (Tell, 2005).

In avian species, clinical manifestations generally depend on the infective dose, spore distribution, preexisting diseases, and immune response of the host (Seyedmousavi *et al.*, 2015). The disease presents in two main clinical courses, acute and chronic. The acute form usually occurs when an overwhelming number of spores are inhaled by an immunocompromised host. The course is rapid, usually less than one week. The chronic form is more common. It can be either localized or systemic and often causes a progressive and debilitating illness that leads to death (McMillan and Petrak, 1989). Therefore, the present report describes the epidemiologic, clinical, anatomopathological, immunohistochemical and microbiological findings of aspergillosis in domestic and wild birds in northeastern Brazil.

MATERIAL AND METHODS

A retrospective study was carried out in all postmortem examinations performed on birds, from January 2000 to December 2022, at the Animal Pathology Laboratory of the Federal University of Campina Grande, Patos, Paraíba, northeastern Brazil.

Epidemiological data, clinical signs and gross lesions were reviewed from the reports. Samples of the skin, central nervous system, and organs within the coelomic cavity were fixed in 10% buffered formalin, processed routinely, embedded in paraffin wax and cut into 3 µm sections. The sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Grocott's methenamine silver stain (GMS).

For culture and mycological identification of *Aspergillus* spp. the samples were put into dextrose Sabouraud agar plates, added with 0.05 g/L of chloramphenicol, and incubated at 37-40° C for seven days. Direct examination of the colonies was performed by placing a filament of fungal culture between the slide and coverslip with a sterile loop and staining with lactophenol cotton blue. The slides were observed at a magnification of 100 to 400x by light microscopy.

The immunohistochemistry (IHC) technique was performed to detect *Aspergillus* spp. using the primary monoclonal antibody, made in mouse, anti-*Aspergillus* spp. (Clone WF-AF-1, AbD Serotec). Briefly, the IHC protocol was as described below. Nonspecific reactions were blocked with 5% casein (skimmed milk powder) for 30 minutes at room temperature. Antigen retrieval was performed with Tris-EDTA (pH 9.0) in microwave for 10 minutes. The primary antibody was incubated at 1:300 dilution for 1h at 37C. Then, biotinylated secondary antibody and streptavidin-biotin-alkaline phosphatase complex (LSAB+System AP; Dako) were used. The substrate-chromogen was Liquid Permanent Red (Dako). Sections were counterstained with Mayer's Hematoxylin. As a positive control, a tissue section from a previously tested and positive case for aspergillosis was utilized. As a negative control, sections of the analyzed tissues were used and incubated only with the antibody diluent (PBST).

RESULTS

During the study period, 357 postmortem examinations were performed on avian species. Among these, 16 (4.48%) cases of aspergillosis were diagnosed. The disease affected domestic and wild birds in the form of sporadic cases and an outbreak. The main epidemiologic and clinical findings were summarized in Table 1.

Table 1. Epidemiologic and clinical findings of aspergillosis in birds in northeastern Brazil.

Case	Year	Species	Affected/Flock	Age	Sex	Rearing system	Clinical aspects		
							Clinical form	Main clinical signs	Course
1	2006	Amazon parrot (<i>Amazona aestiva</i>)	01	Adult	M	Domiciliated	Digestive	Anorexia, weight loss and dysphagia	Undefined
2	2008	Magellanic penguin (<i>Spheniscus magellanicus</i>)	01	Adult	M	Free-living	Respiratory	Anorexia, dehydration, lethargy, weight loss, wheezes and dyspnea	3 weeks
3	2014	Quail (<i>Coturnix coturnix</i>)	09/240	<60D	F/M	Intensive	Respiratory	Cachexia and dyspnea	+ 1 week
4	2017	Domestic chicken (<i>Gallus gallus domesticus</i>)	01/05	<1Y	M	Extensive	Respiratory	Cachexia, dyspnea, wheezing, and periocular swelling	+ 2 weeks
5	2018	Domestic chicken (<i>Gallus gallus domesticus</i>)	01/20	Adult	M	Extensive	Multisystemic	Anorexia, pale mucous membranes, cachexia, ataxia, reluctance to move, paresis of limbs	+ 3 weeks
6	2018	Domestic chicken (<i>Gallus gallus domesticus</i>)	01/30	Adult	M	Extensive	Respiratory	Anorexia, weight loss, reluctance to move	1 week
7	2019	Amazon parrot (<i>Amazona aestiva</i>)	01	Elderly	M	Domiciliated	Multisystemic	Weakness, sneezing, wheezes and falls	+ 1 month
8	2020	Domestic chicken (<i>Gallus gallus domesticus</i>)	01/14	<1Y	F	Extensive	Multisystemic	Apathy, anorexia, weight loss, lateral recumbency and opisthotonos	3 weeks

D: Days. Y: Year. F: female. M: male.

The sporadic cases affected mainly domestic chickens (04/16), followed by wild Amazon parrots (02/16), and a Magellanic penguin (01/16). The birds were adults (04/16), young adults (02/16) or elderly (01/16); predominantly males (06/16); and reared in an extensive (04/16) or domiciliated (02/16) systems, except for the penguin that was free-living (01/16) and was rescued in poor conditions for a shelter, but did not recover and died weeks later.

The outbreak affected domestic quails (09/16), 30-60 days old, of both sexes and reared intensively since the first day of life. The birds were experimentally fed with different amounts

of apple of sodom (*Calotropis procera*) to evaluate the nutritional potential of the food. Of a total of 240 quails, 120 became ill, 90 died and 09 were referred for postmortem examination presenting a cachectic body condition.

Affected birds showed a clinical course ranging from acute to chronic, considering an interval of one week to more than one month. Although in some cases it was not possible to determine the precise evolution of the disease. Three main clinical forms were noted: respiratory (12/16), multisystemic (3/16), and digestive (1/16). Necropsy revealed lesions primarily in the respiratory system (15/16), characterized by

white to yellow caseous nodules, 0.2–5 cm in diameter, mainly distributed in the thoracic (cranial and caudal) and abdominal air sacs, but also found in the cervical and interclavicular air sacs (Fig. 1A and B). The walls of the air sacs were sometimes thickened, opaque and with multifocal areas of adhesion (Fig. 1C). In some cases (08/16) there were yellow caseous lesions that ranged from miliary to large nodules on the subpleural surface of the lungs. At cut, areas of yellowish and granular caseous exudate were noted (Fig. 1D).

In some cases, in addition to the respiratory tract, there were lesions in other anatomical sites and the infection was considered multisystemic (03/16). Nodular caseous lesions, similar to those seen in the respiratory system, were noted on the serosal surfaces and peritoneum. The liver was enlarged, with densely packed yellow nodules, 0.2–3cm in diameter, over the capsular surface and through the parenchyma (Fig. 1E). In one case, there were lesions in the heart characterized by yellowish firm multifocal to coalescing nodules on the epicardial surface of the right atrium.

In one case (1/16), the lesions were located exclusively on the crop and were characterized by an ulcerated and transmural thickened area, measuring approximately 4 cm in diameter (Fig. 1F).

Histopathological examination revealed granulomatous and heterophilic inflammation associated with fungal hyphae. In the air sacs and lungs, there were large areas of necrosis surrounded by intense inflammatory infiltrates composed of heterophils, lymphocytes, macrophages, and multinucleated giant cells. Sometimes these necrotic areas were partially or completely delimited by a connective tissue capsule, associated with proliferation of fibroblasts, immature collagen fibers, neovascularization, and edema (Fig. 2A and B). Within areas of necrosis and inflammation, numerous negatively stained or weakly basophilic fungal hyphae, and rarely conidiophores, were noted in HE-stained sections

(Fig. 2C). Occasionally, hyphae were also noted in the cytoplasm of macrophages and multinucleated giant cells and invading peripheral blood vessels (angioinvasion). The blood vessel walls were expanded by the aforementioned inflammatory cells, necrotic debris, and fibrin. Pulmonary capillaries were often infiltrated by macrophages associated with fibrin deposition, hemorrhage, and edema.

In the liver, multifocal to coalescent areas of necrosis permeated by inflammatory infiltrates of similar composition and fungal hyphae were noted. Infiltration of macrophages, lymphocytes, and plasma cells in the sinusoidal spaces and vascular congestion was also noted. In the heart, there was a focally extensive area of necrosis associated with inflammation and hyphae. Cardiomyocytes adjacent to the area of lytic necrosis were either swollen with pale vacuolated sarcoplasm or hypereosinophilic shrunken with loss of cross striations, pyknosis or karyolysis. Often, surrounded by fibrin and infiltration of heterophils, macrophages, lymphocytes, plasma cells, and rare multinucleated giant cells.

In the crop, a focally extensive area of necrosis was noted extending from the mucosa to the muscularis layer, permeated by a marked inflammatory infiltrate composed of heterophils and macrophages and occasional lymphocytes and plasma cells, associated with negative profiles of hyphae. In the adjacent areas, there was mild ulceration of the epithelium, infiltration by intact and degenerated heterophils and deposition of a serocellular crust.

The PAS and GMS histochemical stains confirmed the presence of fungal hyphae (Fig. 2D and E), characterized by tubular structures with parallel walls, septations, often branching dichotomously at acute angles, and measuring approximately 3–10 µm, morphologically consistent with *Aspergillus* spp. IHC for *Aspergillus* spp. revealed strong immunolabelling of the cytoplasm and the wall of hyphae in red (Fig. 2F).

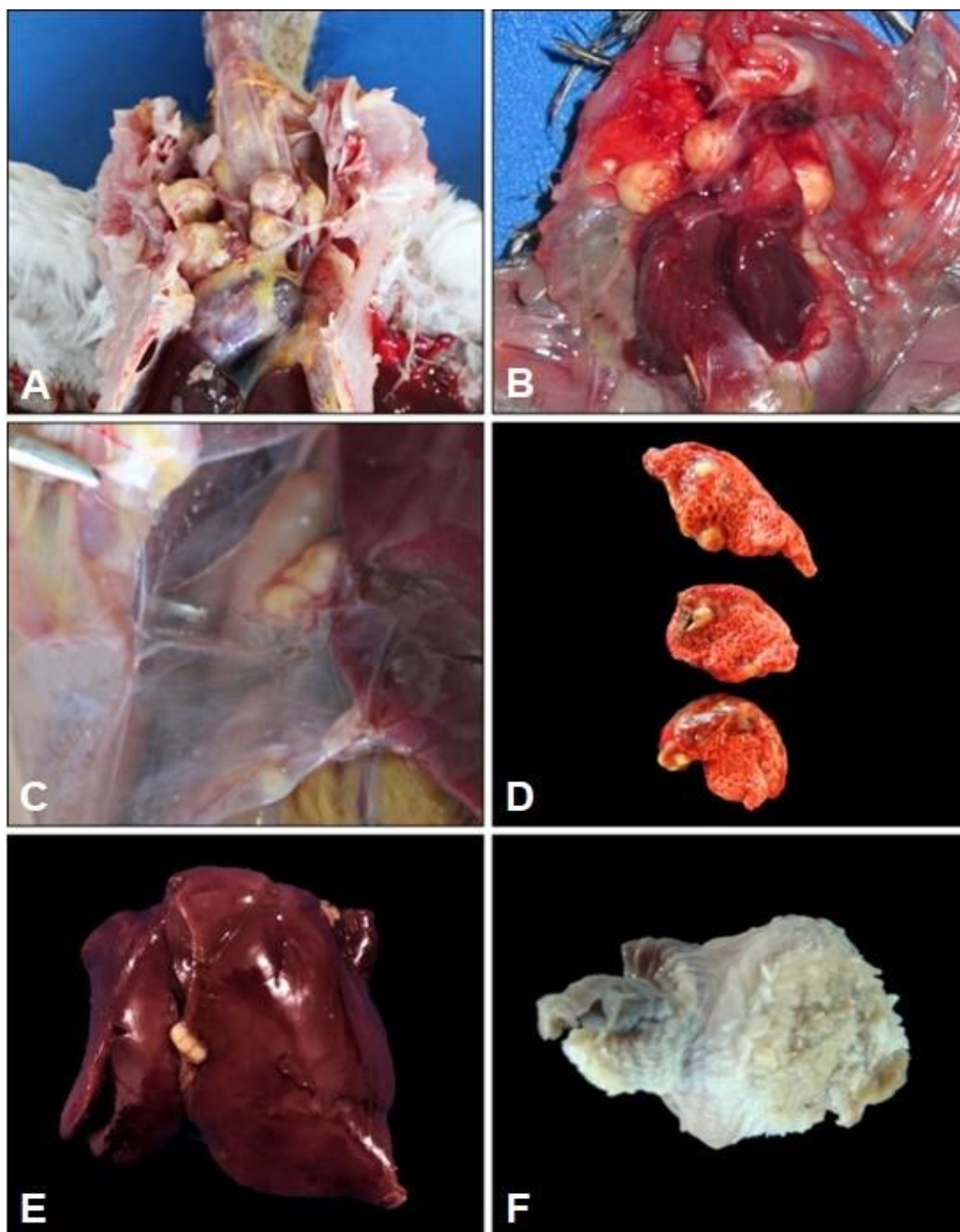


Figure 1. Macroscopic lesions of avian aspergillosis in northeastern Brazil. A) Domestic chicken. Yellow caseous nodules in the cranial thoracic air sacs. B) Domestic quail. Yellow caseous nodules in the caudal thoracic air sacs. C) Amazon parrot. Abdominal air sacs that are thickened, opaque and with yellowish caseous nodules. D) Domestic chicken, lung cross-sections. Multifocal areas of yellowish and granular caseous necrosis. E) Domestic chicken, liver. Slightly enlarged with well-delimited yellowish nodules over the capsular surface. F) Amazon parrot, crop. Focally extensive area of ulceration and transmural necrosis.

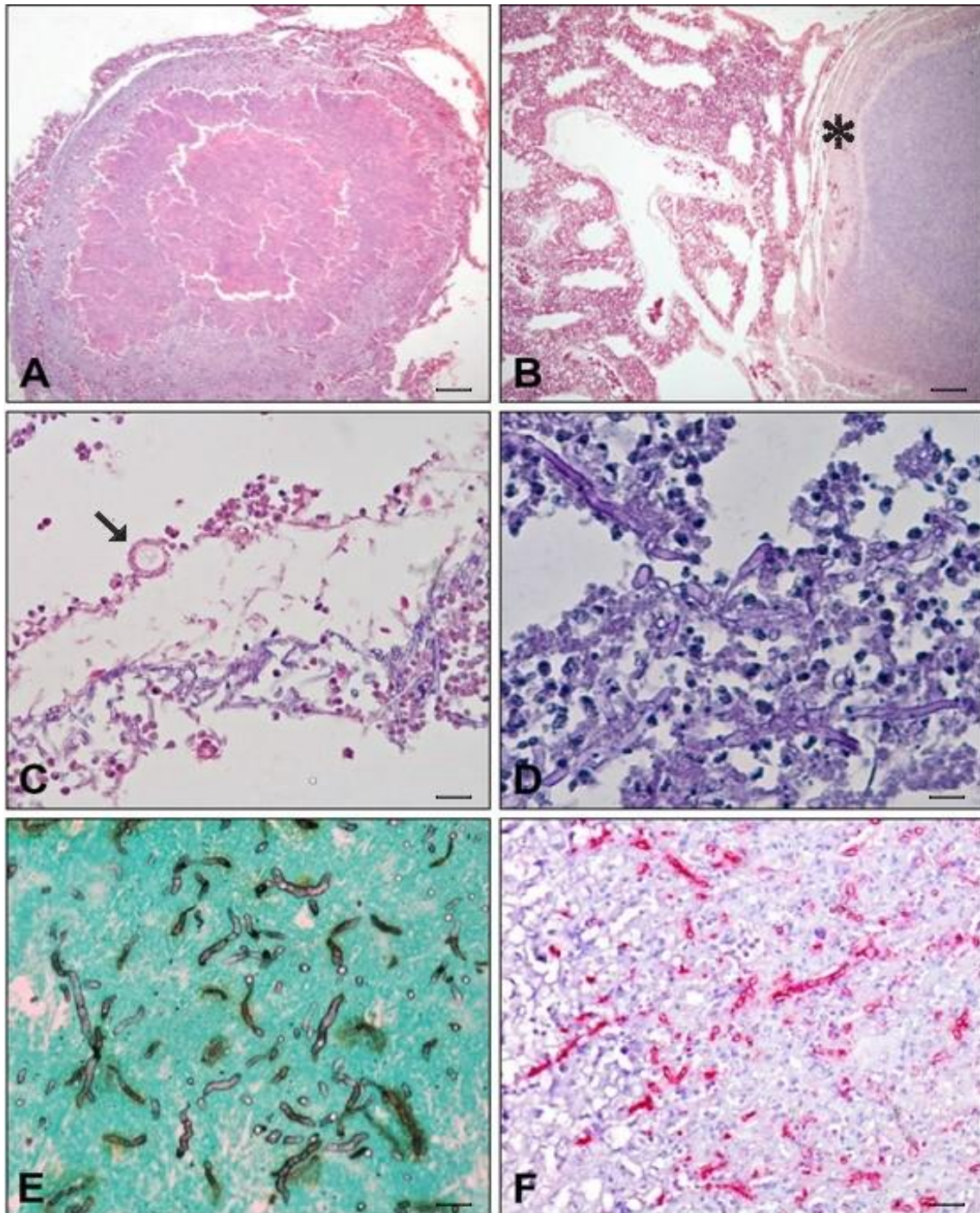


Figure 2. Microscopic lesions of avian aspergillosis in northeastern Brazil. A) Air sac. Central area of necrosis surrounded by intense inflammatory infiltrate. HE. Bar = 5µm. B) Lung. Necrotic core surrounded by inflammatory infiltrate and a connective tissue capsule (asterisk). HE. Bar = 5µm. C) Air sac. Fungal hyphae and conidiophore (asterisk) surrounded by inflammatory infiltrate of heterophils and macrophages. HE. Bar = 40 µm. D) Hyphae intensely stained in blue. PAS. Bar = 40 µm. E) Hyphae intensely stained in black. GMS. Bar = 40 µm. F) Immunolabelling of the hyphae for *Aspergillus* spp. in red. IHC, streptavidin-biotin alkaline phosphatase. Bar = 40 µm.

Mycological cultures yielded pure growth of white fungal colonies that turned greenish and with powdery appearance over time (Fig. 3A). Microscopy revealed fungal hyphae bifurcated at

acute angles, short hyaline conidiophores with a swollen apex (vesicle), metulae, single row of phialides and globose conidia (Fig. 3B).

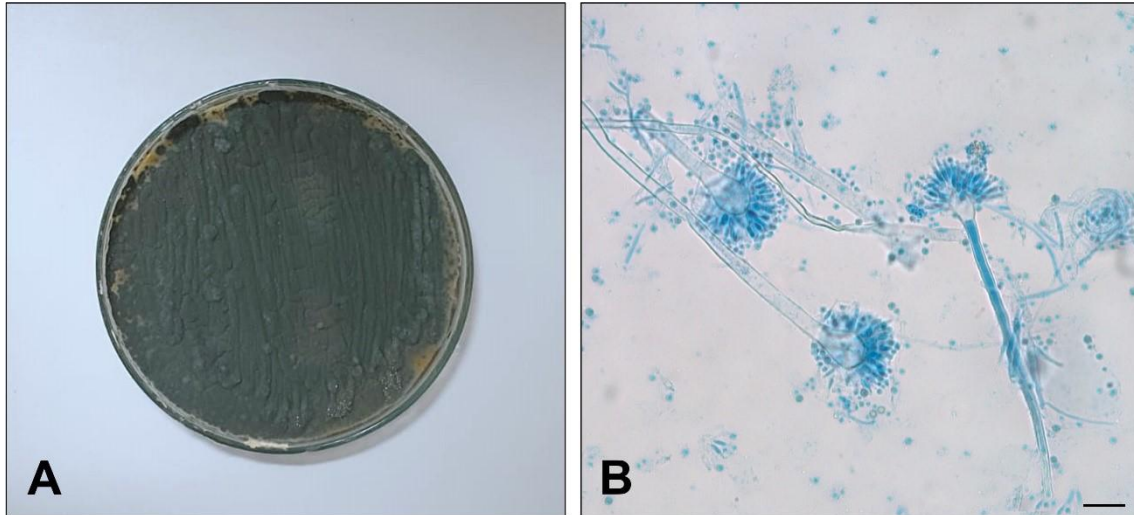


Figure 3. Culture and mycological identification of *Aspergillus* spp. A) Mycology culture. Greenish filamentous fungal colonies characteristics of *Aspergillus* spp. Sabouraud dextrose agar. B) Fungal mycelia and conidiophores with a swollen apex (vesicle), metulae, single row of phialides and globose conidia. Lactophenol cotton blue, 400x.

DISCUSSION

The diagnosis of aspergillosis was based on the epidemiologic, clinical, anatomopathological, immunohistochemical and microbiological findings. Aspergillosis is the most common opportunistic infection of the respiratory tract of birds (Arné *et al.*, 2021). Avian predisposition is attributed to anatomical and physiological factors, primarily related to the respiratory tract. These include absence of a diaphragm and epiglottis, limited distribution of pseudo-stratified ciliated columnar cells, lack of surface macrophages, and mainly the presence of air sacs (Xavier *et al.*, 2011). Characteristics that may suggest that all avian species are susceptible to developing aspergillosis under favorable circumstances (Arné *et al.*, 2021).

Indeed, a relevant finding in this retrospective study was the diversity of bird species affected. Clinical cases have already been diagnosed in chickens (Zamboni *et al.*, 2020; Ceolin *et al.*, 2012), Amazon parrots (McMillan and Petrak, 1989), penguins (Carrasco *et al.*, 2001; Xavier *et al.*, 2011), ostriches (Paixão, *et al.*, 2004), rheas (Copetti *et al.*, 2015), swans (Souza and

Degernes, 2005), and many other bird species. Among the domestic birds, both field data and experimental results have clearly demonstrated a higher susceptibility of quails and turkeys (Vahsen *et al.*, 2021).

Aspergillosis appears to be more significant in conditions of confinement, where predisposing factors like environmental stress, high stocking density, poor ventilation system, inadequate food, and immunosuppression are usually involved (Copetti *et al.*, 2015; Arné *et al.*, 2021). In the outbreak diagnosed in quails, the dietary conditions resulted in malnourished and underdeveloped animals, which certainly determined the impairment of immune responses; in addition to rearing in strict confinement, which may have favored the dispersion of fungal spores.

Nevertheless, the disease also affects domesticated or free-range birds (Beernaert *et al.*, 2010; Arné *et al.*, 2021). Under these conditions, infections tend to occur in the form of sporadic cases, as demonstrated in this survey. Younger birds are more susceptible, especially in the first days of life and when exposed to high

concentrations of *Aspergillus* spp. Conidia (Arné et al., 2021). But elderly birds, with intercurrent illnesses or immunosuppressed by poor husbandry are similarly affected (Seyedmousavi et al., 2015).

The incidence of the disease in males was higher than in females. That unexpected finding was previously noted in another large report of aspergillosis in wild swans (Souza and Degernes, 2005) and a retrospective study in psittacine birds (McMillan and Petrak, 1989), but sex predisposition has not been recognized so far.

The birds presented three main forms of the disease: respiratory, multisystemic, and digestive. Our study supports other descriptions in which the respiratory system is the main site involved (McMillan and Petrak, 1989; Tell, 2005; Beernaert et al., 2010; Della Vedova et al., 2019). However, the lack of antemortem information related to clinical signs and disease progression in some cases prevented us from faithfully characterizing the clinical aspects considering its multiple forms.

The main clinical signs were nonspecific, such as anorexia, weakness and weight loss or even cachexia; but signs directly related to the respiratory tract were also observed, including dyspnea and wheezing. Birds typically do not show clinical signs of illness until the disease is in advanced stages (Souza and Degernes, 2005) and clinical signs clearly depend on which organs are involved (Beernaert et al., 2010).

In these cases, the clinical course ranged from acute to chronic, considering an interval of one week to more than one month. Avian aspergillosis is often classified as acute or chronic (Beernaert et al., 2010). Acute aspergillosis is thought to be the result of inhaling an overwhelming number of spores; while chronic aspergillosis is generally associated with sporadic cases in older birds suffering immune suppression or concomitant diseases (Beernaert et al., 2010; Della Vedova et al., 2019; Arné et al., 2021).

Unfortunately, antemortem diagnosis of avian aspergillosis can be very challenging because none of the available tests are highly sensitive or specific (Souza and Degernes, 2005). Numerous diagnostic tests can be used to aid the diagnosis,

including hematology, plasma biochemistry, serology, aspergillus enzyme-linked immunosorbent assay, radiographic changes, endoscopy, and fungal culture and isolation (Souza and Degernes, 2005; Beernaert et al., 2010). Even so, the results of most of these tests can be considered indicative rather than diagnostic.

Microbiological culture is routinely employed for the diagnosis of aspergillosis in birds, but due to the ubiquitous nature of *Aspergillus* in the environment, positive cultivation from integument or respiratory tissues without any associated lesions should not be interpreted as a positive diagnosis of aspergillosis (Beernaert et al., 2010). It has been described that only a small percentage of the approximately 340 accepted *Aspergillus* species are implicated in the development of avian aspergillosis (Arné et al., 2021). And although the species were not identified in this survey, it is known that *Aspergillus fumigatus* is by far the most prevalent species, representing up to 95% of cases, in both wild and domestic birds (Arné et al., 2021).

Definitive diagnosis requires demonstration of gross lesions and intralesional hyphae through cytology or histopathology (Beernaert et al., 2010). The microscopic lesions are suggestive, but as the hyphae of filamentous fungi have similar histomorphological characteristics, its visualization usually requires confirmation by specificity tests.

Immunohistochemistry is an option to confirm the diagnosis when microbiological culture is not possible or only paraffin-embedded tissues are available, as usually occurs in retrospective studies. Another alternative that has become more routinely available in recent years is extraction, amplification, and sequencing of DNA from paraffin-embedded tissues. Panfungal polymerase chain reaction (PCR), targeting the internal transcribed spacer 2 (ITS-2), is now available to identify fungal and fungal-like microorganisms (Meason-Smith et al., 2017).

The lesions were preferentially located in the respiratory system, mainly in the air sacs and lungs. As infection generally develops following the inhalation of fungal spores, it is believed the air sacs are the primary sites for fungal

adherence and lesion, since the inhaled air first reaches the thoracic and abdominal air sacs prior to contact the lungs (Copetti *et al.*, 2015). Considering the existence of systemic lesions in some cases it is likely that some animals have compromised immune responses. Although, aspergillosis is one of the main causes of mortality in both immunodepressed and immunocompetent birds (Tell, 2005).

In addition to respiratory involvement, lesions were also observed in liver, heart, peritoneum, and serosae, as well as invasion of blood vessels. These findings characterize some of the infections as multisystemic. Furthermore, invasion of blood vessels may be considered as evidence of the hematogenous spread of the fungus (Carrasco *et al.* 2001). Hematogenous or lymphatic dissemination of fungal elements is allowed by hyphal penetration of the lung blood vessels and by means of macrophages carrying viable spores (Arné *et al.*, 2021).

Through histopathology it was possible to identify in the organs of the respiratory system the massive development of the vegetative forms of the fungus, containing many hyphae and rare conidiophores. Periodic acid-Schiff and/or Grocott, Gomori's methenamine silver stains are often used as auxiliary to detect fungal elements in histological sections (Beernaert *et al.*, 2010) and highlight its histomorphological features.

Mycelial development causes tissue necrosis and incites a strong host reaction, typically the development of heterophilic granulomas (Arné *et al.*, 2021). In this respect, an immunocellular factor that might predispose birds to aspergillosis include the dependence on heterophils, which use cationic proteins, hydrolases, and lysosymes rather than myeloperoxidase and oxidative mechanisms for killing fungal hyphae (Harmon, 1998).

Despite the large number of clinical techniques available, none have proven specific, sensitive, and rapid enough for efficient and reliable aspergillosis diagnosis (Savelieff *et al.*, 2018), which poses a problem for timely and accurate aspergillosis treatments. Traditionally, systemic administration of substances within the azoles group, as well as some allylamines and polyene antibiotics, are prescribed to avian patients with aspergillosis (Krautwald-Junghanns *et al.*, 2015).

However, treatment can be difficult because of the limited tolerability and high occurrence of side effects (Krautwald-Junghanns *et al.*, 2015).

CONCLUSION

Aspergillosis affects both domestic and wild birds in the form of sporadic cases and outbreaks, causing significant economic losses in poultry farms. The disease must be included in the differential diagnosis of respiratory, digestive, and multisystemic disorders. The anatomopathological evaluation and fungal culture represent important tools for the diagnosis.

ACKNOWLEDGEMENTS

The authors are grateful to the National Council for Scientific and Technological Development (CNPq) for the postdoctoral scholarship granted to EPFS (CNPq/process number 151086/2022-2) and to the Productivity Scholarship (CNPq/process number 309460/2017-4).

REFERENCES

- ARNÉ, P.; RISCO-CASTILLO, V.; JOUVION, G. *et al.* Aspergillosis in Wild Birds. *J. Fungi.*, v.7, p.241, 2021.
- BEERNAERT, L.A.; PASMANS, F.; VAN WAEYENBERGHE, L. *et al.* Aspergillus infections in birds: a review. *Avian Pathol.*, v.39, p.325-331, 2010.
- CARRASCO, L.; LIMA JR, J.S.; HALFEN, D.C. *et al.* Systemic aspergillosis in an oiled magallanic penguin (*Spheniscus magellanicus*). *J. Vet. Med. B Infect. Dis. Vet. Public Health*, v.48, p.551-554, 2001.
- CEOLIN, L.V.; CORRÊA, I.D.O.; GALIZA, G.J.N. *et al.* Macroscopic and microscopic diagnosis of aspergillosis in poultry. *Acta Sci. Vet.*, v.40, p.1061, 2012.
- COPETTI, M.V.; BARCELOS, A.S.; KOMMERS, G.D. *et al.* Cutaneous, respiratory, and hepatic aspergillosis in Brazilian white Pekin Mallards (*Anas platyrhynchos*). *Mycopathologia*, v.179, p.321-325, 2015.

- DELLA VEDOVA, R.; HEVIA, A.; VIVOT, W. *et al.* Aspergillosis in domestic and wild birds from Argentina. *Braz. J. Vet. Res. Anim. Sci.*, v.56, p.e152460, 2019.
- HARMON, B. Avian heterophils in inflammation and disease resistance. *Poult. Sci.*, v.77, p.972-977, 1998.
- KRAUTWALD-JUNGHANNS, M.E.; VORBRÜGGEN, S.; BÖHME, J. Aspergillosis in birds: an overview of treatment options and regimens. *J. Exot. Pet. Med.*, v.24, p.296-307, 2015.
- LEISHANGTHEM, G.D.; SINGH, N.D.; BRAR, R.S.; BANGA, H.S. Aspergillosis in avian species: a review. *J. Poult. Sci. Technol.*, v.3, p.1-14, 2015.
- MCMILLAN, M.C.; PETRAK, M.L.; Retrospective study of aspergillosis in pet birds. *J. Assoc. Avian Vet.*, v.3, p.211-215, 1989.
- MEASON-SMITH, C.; EDWARDS, E.E.; OLDER, C.E. *et al.* Panfungal polymerase chain reaction for identification of fungal pathogens in formalin-fixed animal tissues. *Vet. Pathol.*, v.54, p.640-648, 2017.
- PAIXÃO, T.A.; NASCIMENTO, E.F.; PARRA, P.N.S.; SANTOS, R.L. Aspergilose em em avestruz (*Struthio camelus*) no Brasil. *Ciênc. Rural*, v.34, p.573-576, 2004.
- SAVELIEFF, M.G.; PAPPALARDO, L. AZMANIS, P. The current status of avian aspergillosis diagnoses: veterinary practice to novel research avenues. *Vet. Clin. Pathol.*, v.47, p.342-362, 2018.
- SEYEDMOUSAVI, S.; GUILLOT, J.; ARNÉ, P. *et al.* Aspergillus and aspergilloses in wild and domestic animals: a global health concern with parallels to human disease. *Med. Mycol.*, v.53, p.765-797, 2015.
- SOUZA, M.J.; DEGERNES, L.A. Mortality Due to Aspergillosis in Wild Swans in Northwest Washington State, 2000-2002. *J. Avian Med. Surg.*, v.19, p.98-106, 2005.
- TELL, L.A. Aspergillosis in mammals and birds: impact on veterinary medicine. *Med. Mycol.*, v.43, Suppl.1, p.S71-S73, 2005.
- VAHSEN, T.; ZAPATA, L.; GUABIRABA, R. *et al.* Cellular and molecular insights on the regulation of innate immune responses to experimental aspergillosis in chicken and turkey poult. *Med. Mycol.*, v.59, p.465-75, 2021.
- XAVIER, M.O.; SOARES, M.P.; CABANA, A.L. *et al.* Clinical and pathological findings of aspergillosis in Magellanic penguins (*Spheniscus magellanicus*). *Ciênc. Anim. Bras.*, v.12, p.520-524, 2011.
- ZAMBONI, R.; ALBERTI, T.S.; SCHEID, H.V. *et al.* Outbreak of avian aspergillosis in colonial-bred chicks (Isa Brown) in southern Rio Grande do Sul - case report. *Arq. Bras. Med. Vet. Zootec.*, v.72, p.1363-1368, 2020.