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Original Article
Livestock Diseases

Cutaneous fungal infections secondary to avian pox in Northeast Brazil[1]

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ABSTRACT.- Silva R.A.F., Olinda R.G., Pimentel L.A., Maia L.A., Frade M.T.S., Kommers G.D., Galiza G.J.N. & Dantas A.F.M. 2023. **Cutaneous fungal infections secondary to avian pox in Northeast Brazil.** *Pesquisa Veterinária Brasileira 43:e07342, 2023.* Programa de Pós-Graduação em Ciência e Saúde Animal, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Av. Universitária s/n, Bairro Santa Cecília, Patos, PB 58708-110, Brazil. E-mail: raquel_fagundesvet@hotmail.com

This study describes the epidemiological and clinical-pathological aspects of outbreaks of avian pox diagnosed in poultry associated with fungal co-infections. A retrospective study was carried out, and cases of avian pox with suspected associated fungal infection were selected. The slides were subjected to routine and special histochemical staining in cases of suspected fungal infection, in addition to performing the immunohistochemical technique. Macroscopically, there were nodular lesions with crust formation, and histologically, the lesions were characterized by hyperplasia of the spinous layer associated with multiple eosinophilic intracytoplasmic inclusions. In seven cases, morphologically compatible structures with fungi were verified through black impregnation with GMS and strongly stained in pink with PAS. The morphotintorial aspects were suggestive of fungi belonging to the genus *Aspergillus* sp. and *Candida* sp. The diagnosis of fungal co-infections was confirmed through immunohistochemistry, with positive immunostaining for fungi of the genus *Aspergillus* sp. (five cases) and *Candida* sp. (two cases). It is concluded that the occurrence of fungal co-infections secondary to the cutaneous lesions of avian pox represents a complicating factor of the disease, favoring the weakness of these animals and death. Therefore, the investigation of associated secondary agents is necessary.

INDEX TERMS: Avian diseases, avipoxvirus, fowlpox, fungal co-infections.

RESUMO.- [Infecções fúngicas cutâneas secundárias à varíola aviária no Nordeste do Brasil.] Este artigo descreve os aspectos epidemiológicos e clínico-patológicos de surtos de varíola aviária diagnosticada em aves domésticas associados a coinfecções fúngicas. Foi realizado um estudo retrospectivo e selecionados os casos de varíola aviária com suspeita de infecção fúngica associada. As lâminas foram submetidas às colorações histoquímicas de rotina e especiais, nos casos de suspeita de infecção fúngica, além de realização da técnica de imuno-histoquímica. Macroscopicamente, haviam lesões nodulares com formação de crostas, e histologicamente, as lesões caracterizavam-se por hiperplasia da camada espinhosa associada a múltiplas inclusões intracitoplasmáticas eosinofílicas. Em sete casos verificou-se a presença de estruturas morfologicamente compatíveis com fungos, através de impregnação em preto por GMS e coradas fortemente em

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rosa por PAS. Os aspectos morfotintoriais foram sugestivos de fungos pertencentes aos gêneros *Aspergillus* sp. e *Candida* sp. O diagnóstico das coinfecções fúngicas foi confirmado através da imuno-histoquímica, com imunomarcação positiva para fungos dos gêneros *Aspergillus* sp. (cinco casos) e *Candida* sp. (dois casos). Através desses dados, pode-se concluir que a ocorrência de coinfecções fúngicas secundárias às lesões cutâneas de varíola aviária representam um fator complicador da doença, favorecendo a debilidade desses animais e óbito. Sendo assim, é necessária a investigação de agentes secundários associados.

TERMOS DE INDEXAÇÃO: Doenças de aves, avipoxívirus, bouba aviária, coinfecções fúngicas.

INTRODUCTION

In Brazil, avian pox is an important disease in extensive poultry farming because it causes a decrease in the production of meat and eggs as a result of the lesions caused in the digestive tract. The morbidity of fowlpox varies according to the health measures adopted, but it has a low mortality rate (Fallavena et al. 1993). Other factors may be related to high death rates in affected animals, such as secondary infections that settle in, thus aggravating the clinical condition (Schoemaker et al. 1998).

Occurrences of secondary infections caused by bacteria and fungus are occasionally described, associated with cutaneous infections of avian pox in different species, triggering superficial or systemic infections (Shrubsole-Cockwill et al. 2010, Echenique et al. 2016). Fungal co-infections and avian smallpox cases have been routinely diagnosed at the Animal Pathology Laboratory of the "Universidade Federal de Campina Grande" (LPA-UFCG). However, there is no study that allows the characterization of these secondary infections related to avian pox. Thus, the objective was to describe cases of fungal co-infection associated with outbreaks of avian pox, characterizing the main epidemiological and clinical-pathological aspects.

MATERIALS AND METHODS

A retrospective study was carried out of cases of necropsies and biopsies of birds at the LPA-UFCG from January 2002 to December 2021. The cases of avian pox were identified, and from these cases of associated fungal infections.

Subsequently, the necropsy and biopsy records of these birds with the diagnosis of avian pox were separated and information related to the epidemiological aspects was obtained, identifying the breed, sex, age, origin of the birds, clinical signs, as well as the macroscopic and microscopic findings described. All histological slides from all cases of avian pox were reviewed and routinely processed for histopathological and stained using the hematoxylin and eosin (HE).

Special histochemical techniques of Grocott's Methenamine Silver Nitrate (GMS) and Periodic Acid-Schiff (PAS) were applied in cases with suspected secondary fungal infection to observe the morphological characteristics. The immunohistochemistry (IHC) technique was performed on 3µm thick tissue sections on silanized slides, which were deparaffinized and rehydrated. Endogenous peroxidase was blocked with hydrogen peroxide (3% commercial hydrogen peroxide) for 2x10 minutes. Antigenic retrieval was performed in a microwave oven at maximum power with TRIS-EDTA (pH 9.0) for 10 minutes. Nonspecific reactions were blocked with casein (5% skimmed milk powder) at room temperature (25°C) for 30 minutes. The primary antibodies used were: I) monoclonal antibody, produced in mice, anti-*Aspergillus* spp. (Clone WF-AF-1; AbD Serotec) diluted 1:300 in PBST (phosphate saline buffer with Tween-20); and II) polyclonal antibody, produced in rabbit, anti-*Candida albicans* (Abcam; ab53891), diluted 1:3000 in PBST. The sections were incubated with the respective primary antibodies in an oven at 37°C for 60 minutes. As a secondary antibody, the HiDef-HRP polymer detection system (Cell Marque; Millipore-Sigma) was used, as well as the substrate-chromogen 3,3' diaminobenzidine (DAB; EasyLink One Kit [EasyPath]). Sections were counterstained with Harris hematoxylin and mounted with a synthetic mounting medium (Entellan). As a negative control, the primary antibody was replaced with PBST. As a positive control, tissue sections diagnosed with aspergillosis and candidiasis were used.

RESULTS

During the study period, 337 examinations were performed in birds, of which 314 were necropsied and 23 biopsied. Fourteen avian pox outbreaks were diagnosed, some animals died, and others were euthanized due to unfavorable clinical conditions. In seven cases of these outbreaks, secondary fungal infections associated with lesions caused by the avipoxvirus were observed.

In the cases studied, the occurrence was observed in birds reared in the extensive system, affecting animals aged between 15 and 30 days, males and females, of mixed breed. Clinical signs ranged from apathy (10/14), weight loss (7/14), and proliferative lesions on the face, beak and around the eyes (Fig.1 and 2). Skin lesions were sometimes seen on feathered regions such as the chest and abdomen. The time of occurrence of cases was between the months of May and November. The months of September and October stood out for the highest incidence, where there were two cases in each month.

Macroscopic and microscopic findings were similar in all cases. Macroscopic lesions affected the head and wing tips. They were characterized by crusted, nodular and proliferative lesions raised on the face, mainly affecting the beak, cere and around the eyes, and various regions such as the abdomen and pectoral region. Histological lesions were characterized by hydropic degeneration of keratinocytes with markedly hyperplastic epidermis associated with multiple eosinophilic intracytoplasmic inclusions measuring approximately 10 to 30µm in diameter. In the superficial dermis, there was necrosis and a discreet inflammatory infiltrate consisting of heterophils, macrophages and multinucleated giant cells surrounding the dermis' necrosis areas. No microscopic lesions were observed in the other organs.

In seven cases, structures morphologically compatible with fungi were found in the HE. Parallel hyphae with weakly basophilic walls or negative and septate images measuring eight in diameter were predominantly observed. The cytoplasm of the hyphae was filled with basophilic material (Fig.3). Yeasts were characterized by rounded basophilic structures measuring 3-8µm in diameter, sometimes surrounded by a discrete clear halo. Hyphae appeared as negative tubular images with parallel walls, measuring 4 to 12µm, rarely septate and sometimes with slightly eosinophilic cytoplasm (Fig.4). In five cases, the GMS stain hyphae impregnated with black, septate, angular and dichotomous, suggestive of *Aspergillus* sp. (Fig.5).

In two cases, the GMS stain black impregnated hyphae with wide, irregular walls and rare septations, numerous yeasts suggestive of *Candida* sp. (Fig.6). In all cases are strongly stained in pink in the PAS (Fig.7 and 8).

In immunohistochemistry, the hyphae were immunostained with anti-*Aspergillus* and anti-*Candida albicans* antibodies, respectively (Fig.9 and 10).

DISCUSSION

The diagnosis of avian pox was based on epidemiological, clinical and pathological data and confirmed by the presence of structures morphologically compatible with intracytoplasmic inclusions in keratinocytes on microscopy. The diagnosis of fungal co-infection was made according to the histomorphological and staining characteristics of the hyphae, pseudohyphae and yeasts, and confirmed by immunohistochemistry. Secondary infections caused by opportunistic bacteria and fungi have been the most common complications of the cutaneous form of fowlpox (Schoemaker et al. 1998). In a total of fourteen outbreaks of avian pox, seven cases were observed with secondary involvement caused by fungi, demonstrating a high incidence of these secondary infections.

The age range of affected animals is related to the immune deficit in young animals, contributing to the appearance of skin lesions and subsequent secondary infection (Tripathy & Reed 2008). Avian pox usually has a low mortality rate; in most cases, it can be self-limiting (Silva et al. 2009). However, mortality can be high in cases associated with secondary

Fig.1-2. Fungal co-infections and avian pox in birds. Skin, nodular and crusted, multifocal to coalescent lesions are observed in the head region.

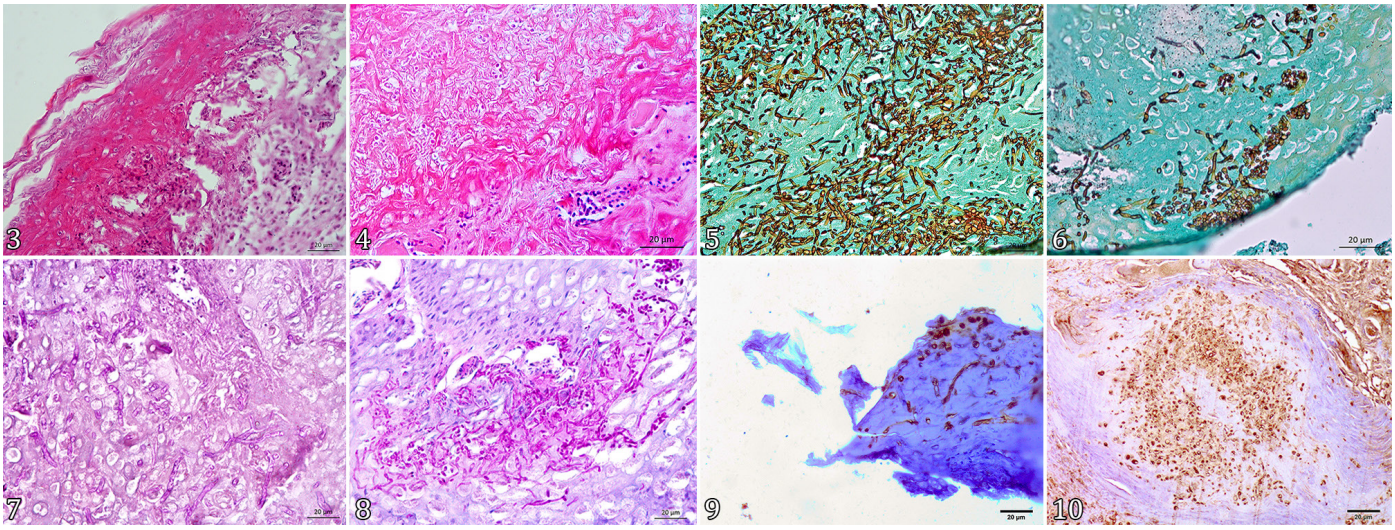

Fig.3-10. (**3**) Skin, fungal structures were observed, predominantly parallel hyphae with weakly basophilic walls or negative and septate images, measuring 8 in diameter. The cytoplasm of the hyphae was filled with basophilic material. HE, bar = 20µm. (**4**) Skin, yeasts and hyphae were identified in large amounts in the lesions. Yeasts were characterized by rounded basophilic structures measuring 3-8µm in diameter, sometimes surrounded by a discrete clear halo. Hyphae appeared as negative tubular images with parallel walls, measuring 4 to 12µm, rarely septate and sometimes with slightly eosinophilic cytoplasm. HE, bar = 20µm. (**5**) Skin, hyphae impregnated with black, septate, angular and dichotomous, suggestive of *Aspergillus* sp. GMS, bar = 20µm. (**6**) Skin, black impregnated hyphae with wide, irregular walls and rare septations, and numerous yeasts suggestive of *Candida* sp. GMS, bar = 20µm. (**7** and **8**) Skin, hyphae and yeasts were intensely stained pink by PAS, bar = 20µm. (**9**) Hyphae immunostained for the monoclonal antibody anti-*Aspergillus* spp. IHC, DAB, bar = 20µm. (**10**) Hyphae immunostained for the monoclonal antibody anti-*Candida albicans* IHC, DAB, bar = 20µm.

fungal infections (Schoemaker et al. 1998, Berchieri Júnior & Macari 2000).

These opportunistic infections occur secondary to avian pox skin lesions due to the port of entry, requiring ulceration for colonization and fungal proliferation to occur. Due to the viral infection, vesicles are formed that later rupture and expose the epidermis to infections by these agents (Hansen 1999).

The outbreaks occurred between the warmest months, which may be related to the increase in arthropod population, such as mosquitoes and ectoparasites that transmit avian pox (Zylberberg et al. 2013). In extensive farming systems, environmental conditions make it difficult to properly disinfect farms, contributing to the constant presence of the virus and the possible emergence of the disease (Revolledo 2009).

In the outbreaks presented, the macroscopic and microscopic findings of the cutaneous form of avian pox are similar to those described in the literature. Proliferative lesions are observed in the skin of regions devoid of feathers; however, they are rarely observed in feathered regions (Tripathy & Reed 2008, Revolledo 2009, Vargas et al. 2011). Fungi of the genus *Aspergillus* sp. and *Candida* sp. were found concomitantly associated with avian pox lesions, characterizing themselves as superficial cutaneous mycoses. The clinical presentation as a superficial cutaneous mycosis differs from what is usually observed in cases of infection by these agents due to its angioinvasive character, which contributes to systemic involvement in birds (Ceolin et al. 2012, Barathidasan et al. 2013). Secondary fungal infections caused by *Candida* sp. can induce serious lesions in Lovebirds (*Agapornis roseicollis*) and Golden Eagles (*Aquila chrysaetos*), leading to pulmonary granulomas and encephalitis (Tsai et al. 1997, Shrubsole-cockwill et al. 2010).

In Brazil, a case of infection by *Aspergillus fumigatus* associated with the avian pox virus was described in an owl (*Bubo virginianus*), in which disseminated lesions were observed that contributed to the weakness and death of the animal (Echenique et al. 2016). Aspergillosis associated with fatal systemic poxvirus infection has been described in canaries, with fungi of the genus *Aspergillus* predominantly found in infected birds (Reza et al. 2013).

The use of antifungals is recommended to prevent or combat fungal infections, thus reducing the severity of clinical signs in affected animals. Several drugs can be used to treat mycoses, such as amphotericin B, fluconazole, itraconazole and ketoconazole. However, caution is recommended in the use of antibiotics due to changes in the microbiota that may favor infection by fungi of the genus *Candida* sp. (Beernaert et al. 2010).

CONCLUSION

The occurrence of fungal co-infections secondary to the cutaneous lesions of avian pox represents a complicating factor for the disease, since the affected birds can easily evolve to death, thus causing significant economic losses for producers.

Conflict of interest statement.- The authors declare that they have no conflicts of interest. The authors are solely responsible for the content and writing of this manuscript.

REFERENCES

Barathidasan R., Singh S.D., Saini M., Sharma A.K. & Dhama K. 2013. The first case of angioinvasive pulmonary aspergillosis in a Himalayan Griffon Vulture (*Gyps himalayensis*). Avian Biol. Res. 6(4):302-306. <https://dx.doi.org/10.3184/175815513X13818257354160>

Beernaert L.A., Pasmans F., Van Waeyenberghe L., Haesebrouck F. & Martel A. 2010. Aspergillus infections in birds: a review. Avian Pathol. 39(5):325-331. <https://dx.doi.org/10.1080/03079457.2010.50621039(5)> <PMid:20954008>

Berchieri Júnior A. & Macari M. 2000. Doenças das Aves. 2ª ed. Facta, Campinas, p.333-338.

Ceolin L.V., Corrêa I.M.O., Galiza G.J.N., Lovato M., Kommers G.D., Risso N. & Santurio J.M. 2012. Diagnóstico macro e microscópico de Aspergilose em frangos de corte. Acta Scient. Vet. 40(3):1061.

Echenique J.V.Z., Bandarra P.M., Brauner R.K., Soares M.P., Coimbra M.A.A. & Schild A.L. 2016. Infecção por pox vírus e *Aspergillus fumigatus* em *Bubo virginianus* (Coruja jacurutu). Pesq. Vet. Bras. 36(7):630-633. <https://dx.doi.org/10.1590/S0100-736X2016000700012>

Fallavena L.C., Rodrigues N.C., Scheufler W., Martins N.R., Braga A.C., Salle C.T. & Moraes H.L. 1993. Atypical fowl pox in broiler chickens in southern Brazil. Vet. Rec. 132(25):635. <https://dx.doi.org/10.1136/vr.132.25.635> <PMid:8394610>

Hansen W. 1999. Avian pox, p.163-169. In: Friend M. & Franson J.C. (Eds), Field Manual of Wildlife Diseases: general field procedures and diseases of birds. USGS, Wisconsin.

Revolledo L. 2009. Bouba aviária, p.154-57. In: Revolledo L. & Ferreira A.J.P. (Orgs), Patologia Aviária. Manole, São Paulo.

Reza K., Nasrin A. & Mahmoud S. 2013. Clinical and pathological findings of concurrent poxvirus lesions and aspergillosis infection in canaries. Asian Pac. J. Trop. Biomed. 3(3):182-185. <https://dx.doi.org/10.1016/S2221-1691(13)60046-5> <PMid:23620834>

Schoemaker N.J., Dorrestein G.M. & Lumeij J.T. 1998. An avipoxvirus infection in a goshawk (*Accipiter gentilis*). Avian Pathol. 27(1):103-106. <https://dx.doi.org/10.1080/03079459808419282> <PMid:18483972>

Shrubsole-Cockwill A.N., Millins C., Jardine C., Kachur K. & Parker D.L. 2010. Avian pox infections with secondary *Candida albicans* encephalitis in a juvenile golden eagle (*Aquila chrysaetos*). J. Avian. Med. Surg. 24(1):64-71. <https://dx.doi.org/10.1647/2009-010R.1> <PMid:20496607>

Silva P.S., Batinga T.B., Sales T.S., Herval E.F.G., Ramos I., Maia P.C.C. & Fernandes L.M.B. 2009. Fowlpox: identification and adoption of prophylactic measures in backyard chickens in Bahia, Brazil. Braz. J. Poult. Sci. 11(2):115-119. <https://dx.doi.org/10.1590/S1516-635X2009000200007>

Tripathy D.N. & Reed W.M. 2008. Pox, p.291-304. In: Saif Y.M. (Ed.), Disease of Poultry. 12th ed. Blackwell, Iowa.

Tsai S.S., Chang T.C., Yang S.F., Chi Y.C., Cher R.S., Chien M.S. & Itakura C. 1997. Unusual lesions associated with avian poxvirus infection in rosy-faced lovebirds (*Agapornis roseicollis*). Avian Dis. 26(1):75-82. <https://dx.doi.org/10.1080/03079459708419195> <PMid:18483891>

Vargas G.D., Albano A.P., Fischer G., Hübner S., Sallis S.E., Nunes C.F., Raffi M.B. & Soares M.P. 2011. Avian pox virus infection in a common barn owl (*Tyto alba*) in southern Brazil. Pesq. Vet. Bras. 31(7):620-622. <https://dx.doi.org/10.1590/S0100-736X2011000700012>

Zylberberg M., Lee K.A., Klasing K.C. & Wikelski M. 2013. Variation with land use of immune function and prevalence of avian pox in Galapagos Finches. Conserv. Biol. 27(1):103-112. <https://dx.doi.org/10.1111/j.1523-1739.2012.01944.x> <PMid:23082926>