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Airsacculitis - Molds Isolated from Lungs of Broilers from Slaughterhouse under Control of the Federal Inspection Service

Andréia Spanamberg[1], Renata Assis Casagrande[2], Ricardo Araujo[3], David Driemeier[1] & Laerte Ferreiro[1]

ABSTRACT

***Background*:** Molds are filamentous saprophytic fungi with worldwide distribution, which are found in various environmental substrates. Among them, the *Aspergillus* genus stands out as one of the main causes of lung disease. Commercial poultry such as chickens and turkeys, as well as captive and free-ranging wild birds species are most frequently affected. The infection occurs by the inhalation of conidia, which are dispersed in the air by wind. The most frequent acute clinical manifestation is usually observed in young animals, often with episodes of outbreaks in poultry farms, whereas chronic disease is more frequently observed in adult birds. In slaughterhouses, carcass inspection is very important for the detection of certain diseases and for monitoring their occurrence. The objective of this study was to assess the occurrence of aerosacculitis caused by molds in commercial broilers slaughtered in one establishment under Federal Inspection Service (SIF) through mycological, histopathological and molecular diagnosis.

***Materials, Methods & Results*:** The samples (n = 76) came from 9 flocks localized in the Vale do Taquari - State of Rio Grande do Sul, RS, Brazil. Lungs were collected from broilers with airsacculitis, directly from the slaughter line and kept under refrigeration (4ºC). The evaluation and condemnation of carcasses were performed by the sanitary inspection department of the slaughterhouse. The molds were identified as the causal agent through fungal culture, histopathology and PCR analysis. Sixteen (21.0%) lung samples were positive in mycological culture, being *Aspergillus* sections *Fumigati* and *Flavi* the most frequent, and 2 (2.7%) samples showed fungal elements (hyaline, branching septate hyphae). The respective isolates were identified as *Aspergillus fumigatus sensu stricto* following the amplification of β-tub gene fragments and gel electrophoresis. Among the remaining 58 samples: no fungal growth in 61.9% (47/76) cultures and in 14.4% (11/76) were isolated colonies of hyaline septate fungi and of zygomycetes which were considered as common environmental fungi belonged to genera: *Aspergillus, Penicillium, Fusarium, Scopulariopsis, Mucor* and *Rhizopus.*

***Discussion*:** Poultry become infected through aspiration in large numbers of conidia of molds. According to the literature, *A. fumigatus sensu stricto,* which belongs to the section *Fumigati*, is the most ubiquitous in the environment and frequently associated with lung diseases in humans and animals. Identification of fungi to species level is important in order to determine the etiology of the disease and also to detect potential novel agents. Our results corroborate previous studies that showed *A. fumigatus* to be the prevalent etiological agent identified in commercial poultry. Although in smaller quantities, isolates of the section *Flavi* were also recovered from the lungs of healthy chickens. In the present study, only *A. fumigatus* was cultivated from airsacculitis cases. In poultry, many environmental factors may be present and influence the development of aspergillosis, including excessive ammonia and humidity, inadequate temperature and degraded litter. Our results are in agreement with other research where *Aspergillus* was detected in the lungs of chicken condemned by the inspection service. The eco-epidemiology of *Aspergillus* sections *Fumigati* and *Flavi* needs to be reevaluated, especially considering the transmission from environment to animals and humans. Further studies should be conducted in order to correlate clinical aspects with molecular diversity and antifungal profile among *Aspergillus,* as well as, investigate mold diversity isolated from commercial poultry in locations where the poultry industry is economically significant.

Keywords: moulds, *Aspergillus* spp., *Aspergillus* section *Fumigati*, *Aspergillus* section *Flavi*, poultry, airsacculitis.

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[1]Programa de Pós-Graduação em Ciências Veterinárias (PPGCV), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. [2]Programa de Pós-Graduação em Ciência Animal (PPGCA), Universidade do Estado de Santa Catarina (UDESC), Lages, SC, Brazil. [3]Faculdade de Medicina, Universidade do Porto (U.Porto), Porto, Portugal. CORRESPONDENCE: A. Spanamberg [spanamberg.ad@gmail.com]. Faculdade de Veterinária (FaVet) - UFRGS. Av. Bento Gonçalves n. 9090. CEP 91540-000 Porto Alegre, RS, Brazil.

INTRODUCTION

Molds are filamentous saprophytic fungi with worldwide distribution, which are found in various environmental substrates such as soil, grains, seeds, and decomposing vegetation [13]. Among them, the *Aspergillus* genus is one of the main causes of lung disease in birds, and lesions are observed in the air sacs and lungs [4,5].

The disease is caused by the inhalation of conidia, which are asexual fungal propagules spread by wind. Acute disease is usually seen in young birds, often with episodes of outbreaks in poultry farms. Chronic clinical presentation is more often seen in adult animals [18].

In slaughterhouses, carcass examination is very important for the detection of certain diseases and monitoring their occurrence. It is also important for the subsequent follow-up sanitary inspection of the areas from which the batches were originated. The condemnation of carcass due to airsacculitis in poultry is mostly caused by bacterial or viral diseases, but fungi can also be the cause. Few studies in Brazil correlate macroscopic lesions observed in broiler slaughterhouses with the histological findings and pathogens isolated from the injured organs. This diagnostic triad is essential to estimate the real impact of infectious agents as a cause of carcass condemnation [2,16,17].

The objective of this study was to assess the occurrence of aerosacculitis caused by molds in commercial broilers slaughtered in one establishment under Federal Inspection Service (SIF) through mycological, histopathological and molecular diagnosis.

MATERIALS AND METHODS

Samples

The samples (n= 76) came from 9 flocks localized in the Vale do Taquari - State of Rio Grande do Sul (RS). Brazil. Lungs were collected from broilers with aerosacculitis, directly from the slaughter line of one slaughterhouse located in the same region and kept under refrigeration (4ºC). The evaluation and condemnation of carcasses were performed by the sanitary inspection department.

Fungal isolates

Lung tissues were cultured onto Sabouraud Dextrose[1] and Malt Extract Agar[1] plates. After 7 days of incubation (37º to 40ºC) the colonies of hyaline septate fungi were subcultured onto Czapeck-Dox Agar[2] and, colonies of zygomycetes again subcultured onto Sabouraud Dextrose[1], for final macro and microscopical identification. Fungal colonies were selected and maintained in a saline solution stored at 4ºC.

Histopathology

Lungs were collected and fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were stained by Hematoxylin Eosin stain[3] (Histokit).

DNA extraction

The Qiagen DNeasy® plant mini DNA extraction kit[4] (Qiagen) protocol was used to extract DNA only from *Aspergillus* colonies according to manufacturer instructions.

Molecular identification

Molecular identification was performed using specific primers for the identification of *Aspergillus* section *Fumigati* and *A. fumigatus sensu stricto* [16]. Multiplex polymerase chain reaction (PCR) amplification was performed in a total volume of 25 mL containing 1 mL of DNA extract, 12.5 mL Qiagen Taq PCR master mix[4] (Qiagen) and 0.5 mL of each primer (for a 0.2 mMfinal concentration of each primer). The cycling parameters were pre-incubation at 94ºC for 15 min, 35 cycles of denaturation at 94ºC for 30 s, annealing at 69ºC for 90 s, extension at 72ºC for 1 min and a final extension step of 10 min at 72ºC. The amplification products were stained with Blue Green Loading Dye I[5] (LGC Biotecnologia) and subjected to electrophoresis in 2% agarose gel and visualized under ultraviolet light.

RESULTS

The molds were identified as the causal agent through fungal culture, histopathology and PCR analysis. Sixteen (21.0%) lung samples were positive in mycological cultures (Figure 1), being *Aspergillus* sections *Fumigati* and *Flavi* the most frequent. The respective isolates were identified as *Aspergillus fumigatus* following the amplification of β-tub gene fragments and gel electrophoresis. Examination through histopathological analysis of 2 (2/76 = 2.7%) lung samples allowed to observe fungal elements (septate hyaline hyphae with dichotomous branching at acute angles of 45º) compatible with histomorphology of

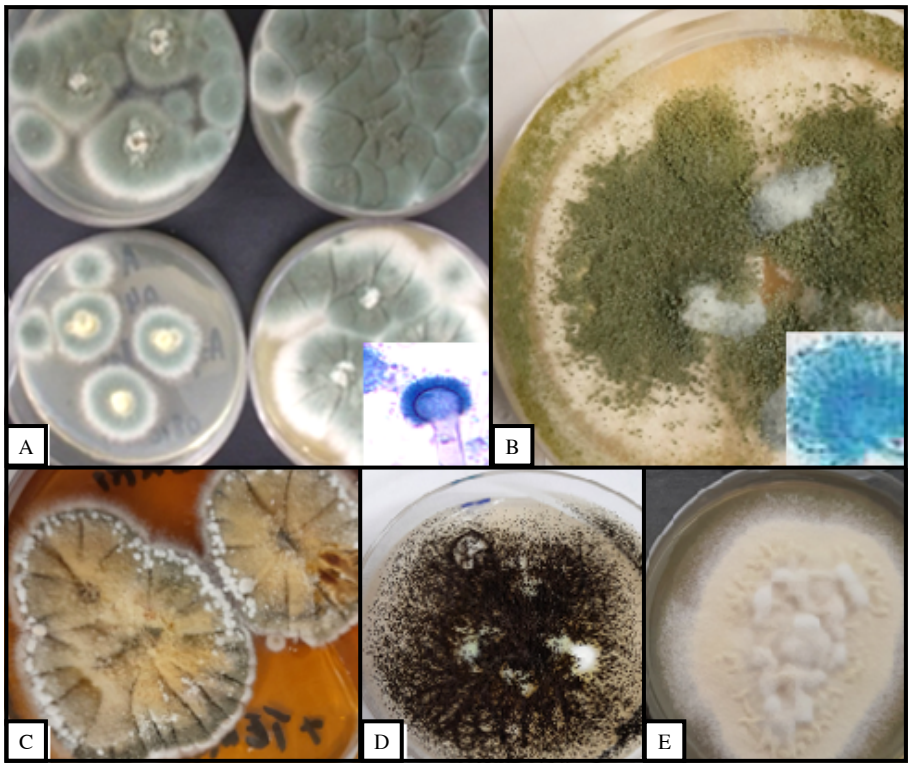

Figure 1. Colonies of common environmental fungi isolates from lungs. A- Four cultures of *Aspergillus fumigatus* (Inset: microscopical features). B- *Aspergillus* section *Flavi* (Inset: microscopical features). C- *Penicillium* sp. D- *Rhizopus* sp. E- *Fusarium*.

Aspergillus spp. Among the remaining 58 samples: no fungal growth in 61.9% (47/76) cultures and in 14.4% (11/76) were isolated colonies of hyaline septate fungi and zygomycetes which were considered as common environmental fungi belonged to genera: *Aspergillus, Penicillium, Fusarium, Scopulariopsis, Mucor* and *Rhizopus.*

DISCUSSION

Aspergillus genus consists of over 440 officially recognized species based on their morphology, physiology, and phylogenetic traits, with implications across various fields such as food production, biotechnology, environmental science, and human / animal health. *Aspergillus* are classified in 6 subgenera, 27 sections and 75 series [6]. According to the literature, *A. fumigatus sensu stricto* is the most ubiquitous in the environment, the most virulent and the main one associated with disease in humans and animals, belonging to the *Fumigati* section. It is followed by *A. flavus* (*Flavi* section) and *A. niger* (*Nigri* section) [6,18,20].

Identification of fungi to species level is important in order to determine the etiology of the disease and also to detect potential novel agents. Our results corroborate previous studies that showed *Aspergillus fumigatus* to be the most prevalent etiological agent identified in commercial poultry [16,17]. *Aspergillus* species are common environmental airborne contaminants; therefore, a positive culture is not proof of infection, being necessary histopathology and molecular tool to confirm the real fungi involvement. Several gene regions, including ITS, and beta-tubulin have been used for the identification of the genera *Aspergillus* [19]. The correct identification of clinical isolates can be done with β-tubulin sequences, without necessity of using sequences of other gene region [8,16,17].

Aspergillus species are found worldwide causing a wide range of diseases from localized infections to fatal disseminated diseases [1,13]. Infections in poultry, commercial and wild birds are frequently pulmonary and also affect the air sacs, lungs and in the coelomic cavity [3]. *Aspergillus* belonging to section *Fumigati* is the main causative agent of aspergillosis in poultry, and is commonly found in bed, soil and decomposing organic matter or in feed. Poultry become infected through aspiration in large numbers of conidia [1]. Most of the isolates in the present study were *A. fumigatus* obtained from broilers with airsacculitis. Similar results were observed by other authors who

demonstrated the dominant association of *Aspergillus fumigatus* with pulmonary avian mycoses [9,12,15].

In poultry, many environmental factors may be present and influence the development of molds. These factors include excessive ammonia and humidity, as well as inadequate temperature and degraded litter [7]. In addition, the growth of toxigenic fungi in the poultry environment may favor the contamination of feed with mycotoxins and/or competing pathogens that may affect avian immunocompetence. *Aspergillus fumigatus* may be present in the lungs of healthy poultry and or may be causing airsacculitis [16,17]. Mortality rates can be high in chicks and turkey poults that inhale large numbers of conidia, possibly while hatching or when placed on bedding contaminated [1,9].

Although in smaller quantities, isolates of *Aspergillus* section *Flavi* were also recovered from the lungs, however, fungal elements were not found in the histopathological examination of the respective samples. Outbreaks of pulmonary aspergillosis caused by *A. flavus* in broiler breeders, turkey, gosling and ostriches are described [1,8,10,11,14]. Our results are in agreement with other research where *Aspergillus* was detected in the lungs of chicken condemned by the inspection service. In slaughterhouses, carcass inspection is extremely important for the detection of diseases and for monitoring their occurrence. Carcass condemnation due to airsacculitis in poultry is mostly caused by bacterial and/or viral diseases [2,8]; however, fungi can also be the [16].

CONCLUSIONS

Aspergillus was detected in aerosacculitis from commercial broilers. The eco-epidemiology of *Aspergillus* sections *Fumigati* and *Flavi* needs to be reevaluated, especially considering the transmission from environment to animals and humans. Further studies should be conducted in order to correlate isolation of potential fungal pathogens with clinical aspects, as well as to investigate the molecular diversity and antifungal profile among *Aspergillus* isolated from broilers where the poultry industry is economically significant. It is also important to know airborne fungi from indoor environment of slaughterhouses.

MANUFACTURERS

[1] Himedia Laboratories Private Limited. Maharashtra, India.
[2] Sigma Aldrich Brasil Ltda. Duque de Caxias, RJ, Brazil.
[3] Erviegas I.C. Ltda. São Paulo, SP, Brazil.
[4] Qiagen. Hilden, Germany.
[5] LGC Biotecnologia. Cotia, SP, Brazil.

***Declaration of interest*.** The authors declare no conflicts of interest. The authors are solely responsible for the content and writing of the article.

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