RESUMO.- [Pesquisa de *Aspergillus fumigatus* em carcaças de frango de corte normais e condenadas por aerossaculite.] Nos abatedouros, a inspeção das carcaças é fundamental para a detecção e monitoramento da prevalência de certas doenças. Os objetivos do trabalho foram avaliar a ocorrência de aspergilose causada por *Aspergillus fumigatus* em aves comerciais através do diagnóstico micológico e histopatológico e verificar a possibilidade de associação causal entre os critérios de diagnóstico de aspergilose e condenação por aerossaculite em frangos de corte através de um estudo de caso-controle. O estudo foi realizado com 380 amostras. Foram coletados pulmões de frangos condenados (95) e não condenados (285) por aerossaculite diretamente da linha de abate de um frigorífico. Quarenta e seis (12%) amostras de pulmão foram positivas na cultura micológica. Do total de amostras, 177 (46,6%) apresentaram alterações histopatológicas, sendo os mais frequentes pneumonia fibrinoheterofílica necrótica, pneumonia heterofílica e hiperplasia linfóide. Do total de 380 pulmões analisados, 65,2% (30) apresentaram al-

**Aspergillus fumigatus** from normal and condemned carcasses with airsacculitis in commercial poultry

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Carcass inspection is important for the detection of certain diseases and for monitoring their prevalence in slaughterhouses. The objective of this study was to assess the occurrence of aspergillosis caused by *Aspergillus fumigatus* in commercial poultry, through mycological and histopathological diagnosis, and to verify the causal association between the aspergillosis diagnosis criteria and condemnation due to airsacculitis in broilers through a case-control study. The study was carried out with 380 samples. Lungs were collected from broilers that were condemned (95) or not condemned (285) due to airsacculitis directly from the slaughter line. Forty-six (12%) lung samples were positive for *A. fumigatus* in mycological culture. Among all samples, 177 (46.6%) presented histopathological alterations, with necrotic, fibrinous, heterophilic pneumonia; heterophilic pneumonia and lymphoid hyperplasia being the most frequent. Out of the 380 lungs analyzed, 65.2% (30) showed histopathological alterations and isolation of fungi. The statistical analysis (McNemar’s chi-square test) indicated a significant association between the presence of histopathological lesions and the isolation of *A. fumigatus*. Mycological cultivation and histopathological diagnosis increase the probability of detecting pulmonary alterations in birds condemned by the Final Inspection System, which suggests that such diagnostic criteria can improve the assessment and condemnation of birds affected by airsacculitis.


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Aspergillosis is one of the main causes of mortality in both immunocompetent and immunodepressed birds. The clinical manifestation of acute aspergillosis is usually observed in young birds, often with episodes of outbreaks in poultry farms, whereas chronic aspergillosis is more frequently observed in adult birds (Tell 2005, Charlton et al. 2008). Its clinical signs depend on the organs or systems involved. The pulmonary system is most frequently affected, with lesions observed in the air sacs and lungs of a wide variety of bird species, which leave the hosts potentially susceptible to infections by *Aspergillus* spp. (Charlton et al. 2008). Clinical cases of aspergillosis have already been diagnosed in chickens (Islan 2009, Ceolin et al. 2012), turkeys (Lair-Fulleringer et al. 2003), ostriches (Paixão et al. 2004), penguins (Carrasco et al. 2001, Xavier et al. 2006), geese (Ziółkowska & Tokarzewski 2007), and many other species (Cray et al. 2009, Spanamberg et al. 2012).

Aspergillosis is most frequently observed in commercial poultry, in which the disease causes stress. This stress is usually associated with poor conditions, such as inadequate ventilation and feed and poultry litter contaminated by large amounts of fungal propagules (Charlton et al. 2008). Several international studies describe the disease both in wild birds and poultry (Islan et al. 2003, Mukaratirwa et al. 2003), ostriches (Cray et al. 2009, Spanamberg et al. 2012), turkeys (Lair-Fulleringer et al. 2003), penguins (Carrasco et al. 2001, Xavier et al. 2006), geese (Ziółkowska & Tokarzewski 2007), and many other species (Cray et al. 2009, Spanamberg et al. 2012).

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The objective of this study was to assess the occurrence of aspergillosis caused by *Aspergillus fumigatus* in commercial poultry, through mycological and histopathological diagnosis. Additionally, this study attempted to verify the possibility of a causal association between aspergillosis diagnosis criteria and condemnation due to airsacculitis in broilers through a case-control study.

### MATERIALS AND METHODS

**Collection of lungs.** The samples (n = 380) came from 56 flocks located in the State of Rio Grande do Sul, RS, Brazil. Lungs were collected from broilers that were condemned (95) or not condemned (285) due to airsacculitis and taken directly from the slaughter line of a slaughterhouse in the above state. The lungs were kept under refrigeration (4°C). The collection and condemnation of carcasses were performed by personnel from the sanitary inspection department. All animal welfare requirements in force were observed in the slaughter process, in accordance with the respective inspection department.

**Anatomopathological Diagnosis.** Part of the lungs (under refrigeration) was sent for mycological examination, and the remaining part was fixed in 10% buffered formalin. These samples were then processed and stained with hematoxylin-eosin (HE) and Grocott (EasyPath®) (Artal 2004).

**Mycological diagnosis.** Lung fragments were streaked onto Sabouraud Dextrose and Malt Extract Agar (37-40°C/7 days) containing chloramphenicol for the isolation of *Aspergillus* spp. The fungal isolates were picked onto Czapeck-Dox agar for final macroscopic and microscopic identification of the species (Stevens 2002).

**Definition of cases and controls.** The lungs of birds condemned due to airsacculitis were defined as "case", and the lungs obtained from normal birds from the same batch were defined as "control". In airsacculitis, total condemnation occurs when widespread lesions are observed in the carcass (emaciation or cachexia), and partial condemnation occurs when the carcass is not impaired (the legs, wings and breast can be used).

**Calculation of sample sizes.** The size of the samples was calculated for an unmatched case-control study, corrected by the Kelsey method (Kelsey et al. 1996). The study was planned for the identification of the odds ratio, with a magnitude of 2 and a power of 80% at a 95% significance level (Kelsey et al. 1996):
Aspergillus fumigatus from normal and condemned carcasses with airsacculitis in commercial poultry

Table 1. Results of mycological culture for Aspergillus fumigatus

<table>
<thead>
<tr>
<th>Mycological culture</th>
<th>Absolute frequency</th>
<th>Relative frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>46</td>
<td>12</td>
</tr>
<tr>
<td>Negative</td>
<td>334</td>
<td>88</td>
</tr>
<tr>
<td>TOTAL</td>
<td>380</td>
<td>100</td>
</tr>
</tbody>
</table>

Forty-six (12%) lung samples were positive for Aspergillus fumigatus in mycological culture (Table 1). These isolates came from 23 flocks located in different counties.

RESULTS

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Forty-six (12%) lung samples were positive for Aspergillus fumigatus in mycological culture (Table 1). These isolates came from 23 flocks located in different counties.

Among all samples, 177 (46.6%) presented histopathological alterations, among which the most frequent were necrotic, fibrinous, heterophilic pneumonia, heterophilic pneumonia and lymphoid hyperplasia (Table 2). Only in one sample were fungal elements detected by HE and Grocott staining (Fig. 1). Fifty-nine (15.52%) lung samples showed macroscopic alterations.

Of the 380 lungs analyzed, 65.2% (30) showed histopathological alterations and isolation of fungi (Table 3). The statistical analysis (McNemar's chi-square test, $\chi^2 = 7.28$, $p<0.05$) indicated a significant association between the presence of histopathological lesions and the isolation of A. fumigatus. Additionally, A. fumigatus was 2.3 times more likely to be isolated in animals with histopathological lesions than in those with no lesions. The logistic regression model identified a significant association of the following assays with condemnation due to airsacculitis (i.e., cases): mycological culture (OR = 11.17; CI$_{95\%}$ = 4.17-30.27; $p<0.001$) and histopathological examination (OR = 3.53; CI$_{95\%}$ = 1.97-6.32; $p<0.001$).

Table 2. Frequency of histopathological alterations (HE staining) in lung samples

<table>
<thead>
<tr>
<th>Histopathologic diagnosis</th>
<th>Lesion intensity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinous heterophilic bronchopneumonia</td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>Necrotic fibrinous heterophilic bronchopneumonia</td>
<td>0 1 1 2</td>
<td></td>
</tr>
<tr>
<td>Fibrinous heterophilic bronchitis</td>
<td>0 3 3 6</td>
<td></td>
</tr>
<tr>
<td>Necrotic fibrinous heterophilic bronchitis</td>
<td>3 2 0 5</td>
<td></td>
</tr>
<tr>
<td>Heterophilic bronchitis</td>
<td>3 0 2 5</td>
<td></td>
</tr>
<tr>
<td>Chronic pleuritis</td>
<td>1 1 0 2</td>
<td></td>
</tr>
<tr>
<td>Fibrinous heterophilic pleuropneumonia</td>
<td>0 1 0 1</td>
<td></td>
</tr>
<tr>
<td>Necrotic fibrinous heterophilic pleuropneumonia</td>
<td>0 0 2 2</td>
<td></td>
</tr>
<tr>
<td>Fibrinous heterophilic pneumonia</td>
<td>1 6 1 8</td>
<td></td>
</tr>
<tr>
<td>Necrotic fibrinous heterophilic pneumonia</td>
<td>0 6 11 17</td>
<td></td>
</tr>
<tr>
<td>Granulomatous pneumonia</td>
<td>0 1 0 1</td>
<td></td>
</tr>
<tr>
<td>Heterophilic pneumonia</td>
<td>13 2 0 15</td>
<td></td>
</tr>
<tr>
<td>Lymphoid hyperplasia</td>
<td>107 22 1 130</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>380</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Histopathological results (HE staining) and Aspergillus fumigatus isolation

<table>
<thead>
<tr>
<th>Mycological culture</th>
<th>Histopathology (HE)</th>
<th>With alterations</th>
<th>Without alterations</th>
<th>Total</th>
<th>Relative frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>30</td>
<td>16</td>
<td>46</td>
<td>65.2</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>147</td>
<td>187</td>
<td>334</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>177</td>
<td>203</td>
<td>380</td>
<td>46.6</td>
<td></td>
</tr>
</tbody>
</table>

* Detection of fungal elements (Grocott staining): hyaline, branching septate hyphae (n=1).

Table 4. Multivariate logistic regression analysis between mycological cultures and histopathology results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate (β)</th>
<th>Standard error</th>
<th>p-value</th>
<th>Odds (CI: 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycological culture</td>
<td>2.41</td>
<td>0.50</td>
<td>&lt;0.001</td>
<td>11.17(4.17-30.27)</td>
</tr>
<tr>
<td>Histopathology (HE)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>With alterations</td>
<td>1.26</td>
<td>0.29</td>
<td>&lt;0.001</td>
<td>3.53(1.97-6.32)</td>
</tr>
<tr>
<td>Without alterations</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
and histopathological testing (OR =3.53; CI₂₀₀₀ =1.97-6.32; p<0.001) (Table 4). The discriminatory power of the model identified by the ROC curve was 70%, and the adequacy of the model was verified using the Hosmer-Lemeshow test (p=0.80).

**DISCUSSION**

The widespread distribution of fungal propagules with tiny diameters, particularly in the case of *Aspergillus fumigatus*, which is found on the anemophilous flora of all continents, constantly exposes the respiratory tract of both humans and animals to fungal colonization.

Tashiro et al. (2011) observed a fungal colonization rate of 45% (62) in 139 human lungs, with *A. fumigatus* being the most frequently isolated fungus (41%). Similarly, Lass-Förl et al. (1999) found that *A. fumigatus* prevailed in 41.07% of 74 positive samples.

In broilers under suspicion of aspergillosis, Sajid et al. (2006) and Islan et al. (2003) isolated *A. fumigatus* in 48.43% and 17.53% of the cultures, respectively. In this study, 46 (12%) of the lungs analyzed contained *A. fumigatus*. Specifically in the case of birds, conditions that favor the development of fungi in confinement buildings expose commercial poultry to a higher risk of inhaling conidia of *A. fumigatus* during the farming period. This is a condition that frequently results in the isolation of the fungus from the lungs of healthy birds (Arné et al. 2011).

Even though only one case was clearly characterized as aspergillosis through histopathological testing and mycological cultivation, we verified that there was a significant association between the two variables. The literature shows that in some cases, cultures of fungi and cytopathological examination of respiratory specimens often yield negative results and a lack sensitivity for detecting fungal elements in an early stage of infection (Tarrand et al. 2003). *Aspergillus fumigatus* is also frequently isolated from straw and straw particles in animal housing for pigs, cattle and poultry, where repeated exposure can cause outbreaks of invasive disease in poultry flocks and respiratory problems in other animals (Arné et al. 2011). Among animals that are affected by aspergillosis, birds are among the species that can naturally acquire infection in the absence of immunodepression (Clemens & Stevens 2005).

A small number of samples with macroscopic alterations (15.52%) was found in the study. In a study of the anatomopathological aspects of aspergillosis in birds, Cacciutolo et al. (2009) suggest that the presence of conidia in their respiratory system may lead to a latent infection without clinical symptoms and macroscopic lesions. Recovery of *Aspergillus* spp. in respiratory samples in the absence of signs of pneumonia suggests that birds may only be carriers of the fungi unless stimulated by a decreased resistance of the host elicited by some stress such as an infectious disease, a toxin or malnutrition (Garcia et al. 2007).

Respiratory infections are common among the chicken population. The histopathological diagnosis of the lung sections showed several alterations in 46.6% of the samples (lymphoid hyperplasia, pneumonia, bronchitis and bronchopneumonia) with variable lesion intensity most likely associated with bacterial and/or viral agents (Zafra et al. 2008). However, our results concur with the findings of Steinlage et al. (2003), which indicate that *A. fumigatus* may not be the primary cause of respiratory infection. The histological alteration observed most frequently was lymphoid hyperplasia. This change is often nonspecific and quite frequently occurs in the respiratory system in response to any injury (Fletcher 2008).

Regarding the logistic regression results, it is possible to suggest that both the mycological cultivation and the histopathological testing increase the probability of detecting pulmonary alterations in birds condemned by the Final Inspection System, which suggests that such diagnostic criteria can improve the assessment and condemnation of birds affected by airsacculitis. Fungal isolation from respiratory samples has been regarded as being of limited usefulness in the *ante mortem* diagnosis of aspergillosis in human patients. However, in livestock, this is a widely used tool for monitoring batches and may point to possible corrective measures for risk factors found in the properties in question.

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