Possible respiratory infection due to *Aspergillus* in workers from swineries and poultries

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INTRODUCTION

Recent epidemiologic studies clearly outline the link between fungal sensitization and exacerbations of asthma, leading to increased morbidity and mortality.

Amongst the filamentous fungi, *Aspergillus* species have been strongly linked with exacerbations of asthma and other respiratory allergic diseases. Particles of approximately 1 to 4 µm are deposited in the lower respiratory tract. Therefore, conidia of *A. fumigatus* are small enough to traverse the terminal respiratory airways and reach the pulmonary alveoli, whereas the larger conidia of some other *Aspergillus* species, such as *A. flavus* and *A. niger*, tend to be deposited in the paranasal sinuses and upper airways (Ben-Ami *et al.*)

Exposure to environmental fungal spores has been associated with worsening asthma symptoms, lung function, hospital admissions and asthma-related deaths.

Materials and methods

Air samples from seven poultries an seven swineries were collected in at 140 L/minute, at one meter tall, onto malt extract agar supplemented with chloramphenicol (MEA). Air collection was also performed outside premises, since this is the place regarded as reference.

Surfaces samples, were collected by swabbing the surfaces of the same indoor places, using a 10 by 10 cm square stencil disinfected with 70% alcohol solution between samples according to the International Standard ISO 18593 – 2004. The obtained swabs were then platted onto MEA. All the collected samples were incubated at 27 ºC for 5 to 7 days.

After laboratory processing and incubation of the collected samples, quantitative (colony forming units – cfu/m^3 and cfu/m^2) and qualitative results were obtained, with identification of the isolated fungal species.

Clinical data: a questionnaire was performed to all the workers from the poultries and swineries analyzed. Demographic and clinical data were recorded and included age, gender, presence of respiratory problems in different situations, and previous asthma diagnosis.

Results and discussion

Aspergillus epidemiology

From the **poultries** analyzed:

✓ 28 different fungal genuses were detected along the study, in a total of 266 isolates.

✓ The prevalence of the *Aspergillus* genus in these settings was 22.2% and ten different species were identified from the 59 collection points from where *Aspergillus* isolates were obtained (**Fig. 1**).

✓ *Aspergillus versicolor* was the most frequent species found (33.9%), followed by *A. flavus* (23.7%) and *A. fumigatus* (15.3%). *Aspergillus flavus*, however, presented the highest level of airborne spores (>2000 cfu/m^3) (**Table 1**).

✓ From the analyzed surfaces, *A. versicolor* was detected in higher number (>3x10^{-2} cfu/m^2). Floor coverage presents the highest count of *Aspergillus* colony forming units (**Table 1**).

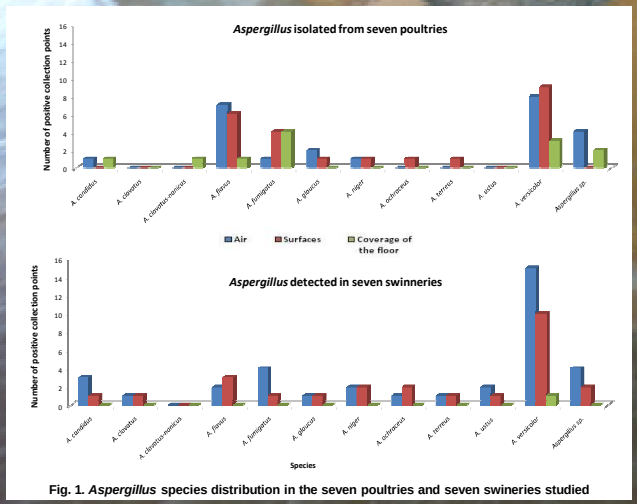


Fig. 1. *Aspergillus* species distribution in the seven poultries and seven swineries studied

From the analyzed **swineries** :

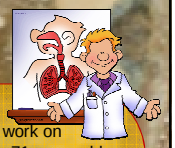
✓ 39 different fungal genuses were detected, in a total of 431 isolates. The prevalence of *Aspergillus* genus in these settings was lesser than in the poultries cases (14.4%).

✓ Twelve different *Aspergillus* species were identified among the 62 collection points from where *Aspergillus* isolates were obtained (**Fig. 1)**

✓ Despite the three most common species being the same as in the previous case, in this setting *A. versicolor* presents the highest airborne spore counts (>2000 ufc/m^3) and the highest overall prevalence (41.9%) (**Table 1**), followed by *A. flavus* and *A. fumigatus* (8.1%). From the analyzed surfaces, *A. versicolor* was detected in higher numbers (>3x10^{-2} cfu/m^2) (**Table 1**).

Table 1. Quantitative results obtained from *Aspergillus* isolation from the air, surfaces and coverage of the floor in the analyzed *settings*

	Poultries			Swineries		
	Air (cfu/m^3) (range)	Surfaces (cfu/m^2) (range)	Coverage of the floor (cfu/g) (range)	Air (cfu/m^3) (range)	Surfaces (cfu/m^2) (range)	Coverage of the floor (cfu/g) (range)
A. candidus	0 - 40	0 -1x10^{-4}	0 - 5x10^4	0 - 60	0 - 1x10^{-4}	0
A. clavatus	0	0 - 2x10^{-4}	0 -1x10^4	0 - 20	0 - 2x10^{-4}	0
A. clavatus-nanicus	0	0	0 - 1x10^3	0	0	0
A. flavus	40 - >2x10^3	0 - >1x10^{-2}	0 – 12.5x10^3	0 - 20	0 - 1x10^{-4}	0
A. fumigatus	0 - 80	0 – 1.1x10^{-3}	0 - 1x10^4	0 -100	0 - 1x10^{-4}	0
A. glaucus	0 - 40	0 -1x10^{-4}	0	0 - 40	0 - 6x10^{-4}	0
A. niger	0 - 80	0 -1x10^{-4}	0	0 - 20	0 - 2x10^{-4}	0
A. ochraceus	0	0 - 2x10^{-4}	0	0 - 20	0 - 6x10^{-4}	0
A. terreus	0	0 -1x10^{-4}	0	0 - 100	0 - 1x10^{-4}	0
A. ustus	0	0	0	0 - 240	0 - 1x10^{-4}	0
A. versicolor	40-960	0 - >3x10^{-2}	0	0 - >2000	0 -3x10^{-2}	0 - 5x10^3
Aspergillus sp.	0 - 220	0	0 - 1x10^4	0 - 40	0 - 6x10^{-4}	0



Clinical data

✓ 80 workers of these settings were analyzed, 47 of them work on poultries and 33 on swineries, with ages ranging from 17 to 71 years old.

✓ 7 workers were previously diagnosed with asthma (2 from poultries and 5 from swineries). 4 of them reported the first attack after 40 years old, suggesting the influence of the working activities in the development of the disease.

✓ A high prevalence of respiratory symptoms in professionals without asthma was observed, namely wheezing associated with dyspnoea (23.8%) and dyspnoea after strenuous activities (12.3%), suggesting an underdiagnosed respiratory problem.

✓ Cough during the night was reported by both asthmatic (42.9%) and non-asthmatic workers (28.8%). Twelve of them (15%) showed to have chest tightness or wheezing at work, which can be directly associated with their involvement in specific activities in the workplace.

✓ 32.5% of all the inquired workers refer an improvement of their respiratory ability during the resting days and holiday (p=0.002), revealing the association of the respiratory disturbances with the working activities.

Concluding Remarks

Considering the strong association between fungal sensitization and severity of asthma and that *Aspergillus* species are one of the most important fungal agents causing sensitization of the individual, this study contributes to the knowledge of *Aspergillus* prevalence and distribution in Portuguese poultries and swineries. Molecular epidemiology of *Aspergillus* species will be performed in further studies. These data, together with data regarding determination of specific IgE levels and studying mycotoxin exposure using specific biomarkers will also help to understand how and which *Aspergillus* can affect the health of workers of these settings.

References

Ben-Ami R, Lewis R, Kontoyiannis, Dimitrios P. (2010). Enemy of the (immunosuppressed) state: an update on the pathogenesis of Aspergillus fumigatus infection. British Journal of Haematology, 150: 406–417.