Feed production, swine and slaughterhouse: where is the highest occupational exposure to *Aspergillus* spp. in this production line?

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Abstract

This study intended to characterize fungal contamination in two swine farms, in one feed production unit, and also in one swine slaughterhouse. We aimed to identify where the highest occupational exposure to *Aspergillus* spp. was detected during the production line. Air and surfaces samples were collected from the four units through impaction and swabbing methods, respectively. Quantitative and qualitative results were obtained, with the identification of the isolated fungal species. For molecular analysis, 300L of air were also collected from each same sampling site using the impinger method. Real Time quantitative PCR (qPCR) was done to perform the molecular detection of the *Aspergillus* sections *Circumdati, Fumigati* and *Flavi* (only the toxigenic strains).

Results: Eleven species of filamentous fungi were identified in air samples from the feed production unit, with a total of 1666 isolates. None *Aspergillus* species were isolated. Seven filamentous fungi species were found in the slaughterhouse, with a total of 810 isolates and only 10 isolates belonging to the *Aspergillus fumigatus* group (section *Fumigati*) were isolated. Twelve fungal species were found in the air from one of the analyzed swine farms in a total of 3080 isolates. *Aspergillus* genus showed low prevalence (6.5%), being the sections *Candidi*, *Circumdati* and *Terrei* the only ones isolated. Regarding the other assessed swine farm, 15 fungal species were identified, in a total of 5080 isolates. *Aspergillus* genus presented the highest prevalence (15.7%). Among this genus, *Eurotium herbariorum* (section *Restricti*) was the most prevalent (45.0%). *Fumigati* and *Circumdati* sections were also isolated. Regarding the results obtained by molecular methods, *A. fumigatus* section was detected in 10 sampling sites where this species-complex was not isolated by conventional methods: 2 in feed production, 4 in slaughterhouse and 2 in each swine farm assessed.

Cultural methods showed that swine farms were the settings with the highest fungal load, presenting also the highest number of isolates belonging to *Aspergillus* spp., and a more diversified number of sections. The applied molecular tools enabled to target selected fungal indicators of higher occupational risk, allowing a more accurate characterization on occupational exposure to *Aspergillus*.

Introduction

Aspergillus is among a growing list of allergens that aggravate asthmatic responses. Significant pulmonary pathology is associated with *Aspergillus*-induced allergic and asthmatic lung disease. Environments with high levels of exposure to fungi are found in animal production. Therefore, farmers working with these are at increased risk for occupational respiratory diseases [1].

In feed production and slaughterhouses high levels of fungal contamination were also found [2-4].

Aim of Study

This study intended to characterize the fungal contamination found in two swine farms, in one feed production unit, and also in one swine slaughterhouse. We aimed to identify where is the highest occupational exposure to *Aspergillus* spp. during the production line.

Materials and Methods

Twenty two air and 22 surfaces samples were collected from the four units through impaction and swabbing methods, respectively. After laboratory processing and incubation of the collected samples, quantitative and qualitative results were obtained, with the subsequent identification of the isolated fungal species.

Fig. 1 - Equipment used for air samples collection

Fig. 2 - Equipment used for air samples collection to apply molecular identification

For molecular analysis, 300L of air were also collected from each same sampling site using the impinger method. Real Time quantitative (qPCR) was done to perform the molecular detection of the *Aspergillus* sections *Circumdati*, *Fumigati* and *Flavi* (only the toxigenic strains).

Results and Discussion

Table 1 shows the fungal burden and, specifically, *Aspergillus* prevalence in the different settings analyzed during this study.

Table 1 – Species of filamentous fungi and the total of isolates, the prevalence of *Aspergillus* and the sections isolated in each local.

Production line	Filamentous fungi	Total of isolates	*Aspergillus* prevalence (%)	Sections
Feed production line	11 species	1666	0	-
Slaughterhouse	7 species	810	1.2	*Fumigati*
Swine Farm 1	12 species	3080	6.5	*Candidi*, *Circumdati* and *Terrei*
Swine Farm 2	15 species	5080	15.7	*Restricti*, *Fumigati* and *Circumdati*

Regarding the results obtained from molecular methods, *A. fumigatus* section was detected in 10 sampling sites negative to this section by conventional methods.

Conclusion

- Cultural methods showed that **swine farms** were the settings with **the highest fungal load**, presenting **also the highest number of isolates** belonging to *Aspergillus* spp., and **more diversified number of *Aspergillus* sections.**
- **Molecular tools** enabled to target selected fungal **indicators of higher occupational risk**, allowing a **more accurate characterization** on occupational exposure to *Aspergillus*.
- **Conventional and molecular methods** showed to be **complementary methodologies** and should always be applied together in occupational environments with high fungal load in order **to ensure the best possible characterization** of fungal burden in a given sampling source.

References

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