ACCESSING FUNGAL CONTAMINATION USING CONVENTIONAL AND MOLECULAR METHODS IN PORTUGUESE POULTRIES

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/ CONTEXT

Epidemiological studies showed an increase prevalence of respiratory symptoms and adverse changes in pulmonary function parameters of poultry workers [1]. It corroborate the increased exposure to risk factors, such as fungal load and their metabolites.

This study aimed to determine the occupational exposure threat due to fungal contamination caused by the toxigenic isolates belonging to the Flavi section and Fumigati section.

/ MATERIALS

- Coriolis µ instrument (Air Sampler Bertin Technologies)
- Coriolis µ sterile cones, 15mL of collection liquid (Bertin Technologies)
- PCR in iQ Real Time Detection System (Bio-Rad)

/ PROTOCOL

Sampling at three different farms inside the facilities and outdoors as a reference (300 L/min, 1 min).

DNA extraction

Detection of toxigenic isolates belonging to the *Flavi* section and *Fumigati* section.

Culture-based analysis was also performed in air, surfaces and litter samples. Incubation of agar plates for 5 to 7 days at +27 ºC.

/ RESULTS

Through Coriolis µ and molecular biology, we were able to detect:

aflatoxigenic strains in pavilions in which Flavi section did not grow in culture.

Fumigati section in one farm that was not identified by culture-based methods.

TABLE 1. Distribution of A. flavus and A. fumigatus species-complex in the collected samples

	Farms	Conventional methodologies (impact method)		Molecular Biology (impinger method)
		Air*b (CFU/m³)	Samples number	
A. flavus species complex *a	1	5800	Identified in: - 3/6 indoor air samples - 3/4 surfaces samples - 1/4 aged litter samples	Detected in 2/4 indoor air samples
	2	-	-	Detected in 2/3 indoor air samples
	3	40	Identified in- 1/1 indoor air samples	Not detected
A. fumigatus species complex	1			Detected in 2/4 indoor air samples
	2	80	Identified in: - 1/3 indoor air samples - 1/3 surfaces samples - 1/3 aged litter samples	Detected in 3/3 indoor air samples
	3	-	Identified in 1/1 new litter samples	Detected in 2/2 indoor air samples

*aAdopted from Viegas et al. 2012; *b Total of colonies

Real-Time PCR was applied only in air samples in our study. With those results, we can suppose that the prevalence of isolates belonging to both *Aspergillus* sections obtained through conventional methods, in surfaces and in litter (new and aged), should be higher than what was detected.

[1] - Radon, K., Danuser, B., Iversen, M., Jorres, R., Monso, E., Opravil, U., et al. (2001). Respiratory symptoms in European animal farmers. European Respiratory Journal, 17, 747–754.

[2] - Viegas, C., Malta-Vacas, J., Sabino, R. (2012). Molecular biology versus conventional methods—complementary methodologies to understand occupational exposure to fungi. International Symposium on Occupational Safety and Hygiene 478 – 479.

/ CONCLUSION

Thanks to the Coriolis® µ, it was possible to characterize the contamination caused by toxigenic strains from *Flavi* section and *Fumigati* section in the poultry units using conventional and molecular methodologies. This study shows the complementarily between cultural and molecular methods in the assessment of occupational exposure to fungi. It raises the concern of occupational threat due to the detected fungal load, but also to the toxigenic potential of these species.

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