



ACCESSING FUNGAL CONTAMINATION USING CONVENTIONAL AND MOLECULAR METHODS IN PORTUGUESE POULTRIES

C. Viegas, J. Malta-Vacas, R. Sabino, S. Viegas & C. Veríssimo

Environmental Health RG, Lisbon School of Health Technology, Polytechnic Institute of Lisbon

/ 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).



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 °C.

/ 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 complementarity 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.

/ RESULTS

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

aflatoxicenic 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/m ³)	Samples number		
<i>A. flavus</i> species-complex	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
<i>A. fumigatus</i> 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

*Adapted from Viegas et al. 2012. ^bTotal 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.

Authorised distributor