Multi-approach sampling protocol to assess occupational exposure to bioburden

Carla Viegas [1,2], Ana Monteiro [1,3,4], Susana Viegas [1,2]

[1] H&TRC- Health & Technology Research Center, ESTeSL- Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa; 1990-094 Lisbon, Portugal;
[2] Centro de Investigação em Saúde Pública, Universidade NOVA de Lisboa, 1600-560 Lisbon, Portugal;
[3] Escola Nacional de Saúde Pública, Universidade NOVA de Lisboa, 1600-560 Lisbon, Portugal;
[4] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, E.N. 10 ao km 139,7, 2695-066 Bobadela LRS, Portugal

For further information please contact: carla.viegas@estesl.ipl.pt

Introduction

Swine production has been associated with health risks and workers' symptoms with an increased prevalence of several respiratory symptoms and diseases, such as chronic bronchitis, chronic obstructive pulmonar disease and organic dust toxicsyndrome[1,2]. In Portugal, as in other countries, large-scale swine production involves several activities in the swine environment that require direct intervention, increasing workers' exposure to organic dust[3].

This study describes an updated protocol for the assessment of occupational exposure to organic dust, to unveil an accurate scenario regarding occupational and environmental risks for workers' health.

Materials and methods

- Active (air samples) and passive sampling methods (surfaces, floor covering and feed samples) were performed (Table 1).
- At each working site, 4 air samples were taken for each media (malt extract agar (MEA), dichloran glycerol (DG18), tryptic soy agar (TSA), Violet Red bile agar (VRBA)
- The molecular detection of the *Aspergillus* sections *Circumdati*, *Fumigati* and *Flavi* (only the toxigenic strains) was performed by Real Time PCR (RT-PCR).

Swine Farms	No. of Air Samples Impaction *	No. of Air Samples Impinger	No. of Surfaces Samples (Walls)	No. of Feed Samples	No. of Floor Cover Samples	Animal Quantity
A	20	5	5	2	1	1768
B	20	5	5	2	1	8000
C	20	4	5	2	1	3300
D	20	5	5	2	1	6000
E	16	4	4	2	1	7000

Table 1. Number of samples collected and animal quantity in each farmere taken for each

Results

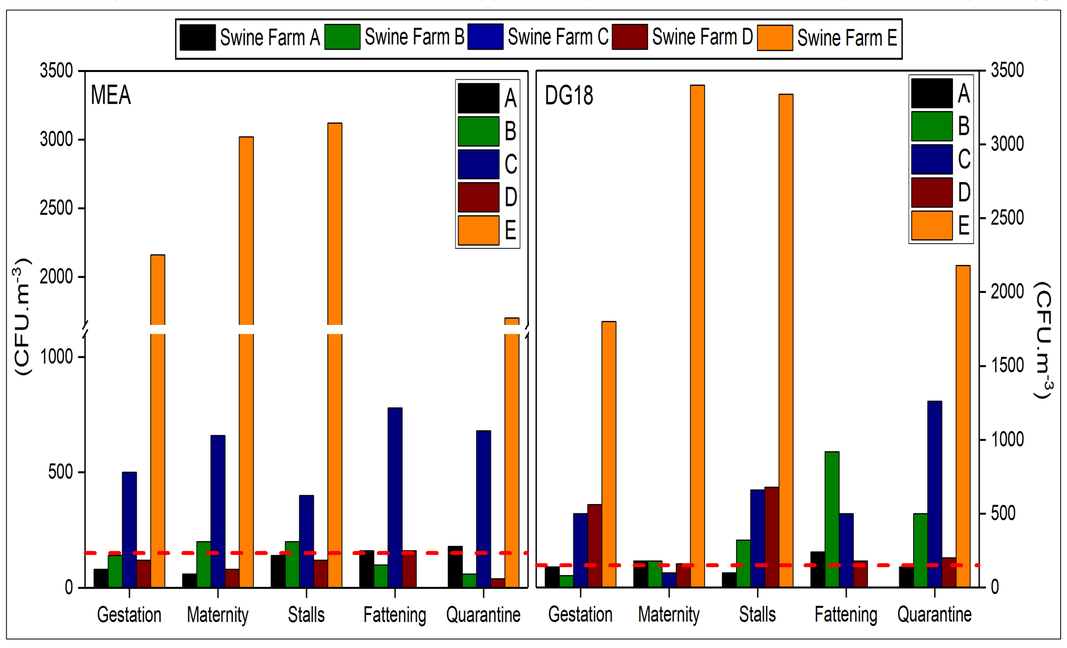
Active methods (Air samples):

Bacteria load: The limit values suggested for total were surpassed in 35.7% (10 out of 28). None of the sampled sites exceeded the limit values suggested for Gram-negative bacteria.

Fungal load: In 65.5% (19 out of 29) of MEA samples surpassed the guideline suggested by World Health Organization (150 CFU.m^{-3}), whereas DG18 revealed an increased amount of sampling sites (82.8%; 24 out of 29) (Figure 1).

Among *Aspergillus* genus found on air samples, section *Circumdati* was the most prevalent (55%) on MEA and *Versicolores* the most identified (50%) on DG18.

Figure 1. Fungal load distribution in the five assessed swine farms [The dashed line represents the reference limits suggested by the World Health Organization (WHO)]



Passive methods (feed, floor coverage and surface swabs)

Results suggest a higher contribution of Gram-positive than Gram-negative bacteria in the **bacteriota load.**

Different **fungal species** were found in the different environmental matrixes assessed and in the different media (Table 2).

No *Aspergillus* section *Fumigati* nor *Aspergillus* section *Versicolores* were detected by qPCR.

Table 2. Fungal distribution in environmental and substrate matrices after inoculation onto MEA and DG18 media.

MEA		DG18	
Air	**(CFU·m^{-3}) (%; n)**	**Air**	**(CFU·m^{-3}) (%; n)**
Cladosporium sp.	59.4; 12,100	*Cladosporium* sp.	66.5; 14,120
Fusarium graminearum	13.2; 2700	*Ulocladium* sp.	14.6; 3100
Alternaria sp.	5.7; 1160	*Chrysonilia sitophila*	4.7; 1000
Others	21.7; 4420	Others	14.2; 3020
Surfaces	**(CFU·m^{-2}) (%; n)**	**Surfaces**	**(CFU·m^{-2}) (%; n)**
Cladosporium sp.	53.8; 210,000	*Scopulariopsis candida*	50.3; 580,000
Scopulariopsis brevicaulis	33.3; 130,000	*Aspergillus* section *Circumdati*	19.9; 230,000
Penicillium sp.	12.8; 50,000	*Cladosporium* sp.	13; 150,000
Others	0.1; 500	Others	16.7; 193,000
Feed	**(CFU·g^{-1}) (%; n)**	***Feed***	**(CFU·g^{-1}) (%; n)**
Cladosporium sp.	71.4; 10	*Cladosporium* sp.	82.2; 37
Penicillium sp.	21.4; 3	*Penicillium* sp.	8.9; 4
Fusarium culmorum	7.1; 1	*Fusarium culmorum*	8.9; 4
Floor covering	**(CFU·g^{-1}) (%; n)**	***Floor covering***	**(CFU·g^{-1}) (%; n)**
Penicillium sp.	50; 4	-	-
Alternaria sp.	37.5; 3	-	-
Cladosporium sp.	12.5; 1	-	-

Discussion and conclusion

The complementarity of the results allows us to infer the importance of using different sampling methods and different culture media to achieve a more accurate exposure assessment [3]. As in other studies developed in settings with high fungal contamination, it was possible to identify one fungal species on surfaces that was not found in air samples[4,5].

The sampling (active and passive) and analysis (culture-based and molecular) methods employed should be adopted as a protocol to be followed in future exposure assessments.

References

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Acknowledgements: This work was supported by the FCT under grant (02/SAICT/2016 – Project nº 23222)