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Horse stable environment: What to expect regarding fungi and particles occupational exposure?

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ABSTRACT: The simultaneous presence of fungi and particles in horse stable environment can create a singular exposure condition because particles have been reported has a good carrier for microorganisms and their metabolites. This study intends to characterize this setting and to recognize fungi and particles occupational exposure. Air samples of 50 L were collected through an impaction method and surfaces samples were also collected by swabbing the surfaces of the same indoor sites. Particles measurements were performed in different tasks using portable direct-reading hand-held equipment. Round pen was the sampling site with the highest fungal load in air and the closed box during litter changing and the feed warehouse has the highest fungal load in surfaces samples. The higher median value for PM_{10} and PM_5 was obtained in the closed box during the horse brushing task ($p < 0.000$). The results obtained in this study highlight the need of further and more detailed research.

1 INTRODUCTION

Bacterial, fungal spores and floor material are the main constituents of respirable dust in stables, being released from fodder and bedding material as well as possibly growing on inner walls due to dampness from horses, especially when washing horses after training (Elfman et al., 2009). Fungi are easily accumulated and aerosolized, acting as indoor air biocontaminants especially in densely horse-populated environments and in enclosed buildings: the consequent exposure may result in respiratory damage (Robison et al.,1996). Besides high fungal contamination there are other risk factors that can be present in this setting such as dust and metabolites produce by fungi (mycotoxins) and bacteria (endotoxins) (Gallagher et al., 2007). The simultaneous presence of these risk factors can create a singular exposure condition because particles have been reported has a good carrier for mycotoxins and endotoxins, promoting exposure to these metabolites (Allermann et al., 2000; Viegas et al., 2013a,b).

Health effects related with exposure to particles exposure in different occupational settings have mainly been investigated with mass-measuring instruments or gravimetric analysis. However, more recently, there are some studies that support that size distribution and particle number concentration may have advantages over particle mass concentration for assessing the health effects of airborne particles (Viegas et al., 2014). It can be an alternative metric that give more detail information regarding the amount of particles that can reach and deposited onto the walls of the respiratory tract (Schmid et al:, 2009).

Many people spend considerable amount of time each day in equine stable environments either as employees in the care and training of horses or in leisure activity. However, to our knowledge, this is the first Portuguese study in this setting that intends to characterize the horse stable environment and to recognize fungi and particles occupational exposure.

2 MATERIALS AND METHODS

2.1 *Equine center assessed*

This study was conducted during June 2014 in one equine center, situated in outskirts of Lisbon city. Samples and measurements were conducted near

the workers nose and during the usual tasks developed in a horse stable. The criteria followed to choose the sampling sites were the ones where the workers spend more time and the tasks that were developed during more time or more frequently in each workplace, namely: closed box when brushing a horse, closed box during litter changing, round pen with horses, uncovered arena with horses, feed warehouse during the handling of feed, covered arena with horses and outdoor to be used as reference. The surface samples were collected in the same sampling sites excluding round pen, arenas and outdoor. The horses were bedded on straw, fed normally three times a day and had their boxes cleaned daily each morning.

2.2 *Fungal contamination*

Sample collections were performed in March of 2014. Air samples of 50 L were collected through an impaction method with a flow rate of 140 L/min onto Malt Extract Agar (MEA) supplemented with chloramphenicol (0.05%), using the Millipore air Tester (Millipore). Surfaces samples were collected by swabbing the surfaces of the same indoor sites, 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 plated onto MEA.

All the collected samples were incubated at 27°C 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. For species identification, microscopic mounts were performed using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures. Morphological Identification was achieved through macro and microscopic characteristics as noted by Hoog et al. (2002).

2.3 *Particles assessment*

In each workplace were identified the tasks usually developed. Based on direct observations of work practices on a task-by-task basis, in the time spend by workers in each one, and in the professional judgment (the task that probably involves higher exposure to particles) it was define the ones to evaluate in each workplace (Table 1).

Measurements were performed using a portable direct-reading hand-held equipment (Lighthouse, model 3016 IAQ). The value of this instrument is its capacity to give information regarding mass concentration in 5 different sizes ($PM_{0.5}$; PM_1; $PM_{2.5}$; PM_5; PM_{10}). Additionally, data related with particle number concentration by each diameter size is also available. In this last case, particles results were given in six different diameters sizes, namely; 0.3 µm, 0.5 µm, 1 µm, 2.5 µm, 5 µm and 10 µm. As mentioned before, this data was also collected because might be more closely correlated with adverse particles health effects (Wichmann et al., 2000).The measurements were conducted near the workers nose and during tasks performance and for each task was done one measurement of approximately 5 minutes.

The data analysis was performed in SPSS statistical software, version 21.0.

Table 1. Tasks assessed in each workplace.

Workplace	N° of tasks	Tasks
Closed boxes	2	Changing litter and cleaning (with and without the horse inside); Horse brushing
Round pen	1	Training horses
Uncovered arena	1	Training horses
Feed warehouse	1	Handling feed
Covered arena	1	Training horses

3 RESULTS

3.1 *Fungal load*

The air results from indoor spaces ranged from 0 $CFU \cdot m^{-3}$ to 100 $CFU \cdot m^{-3}$. Surfaces present higher load than the air with results that ranged from 20000 $CFU \cdot m^{-2}$ to 30000 $CFU \cdot m^{-2}$. Round pen was the sampling site with the highest fungal load in air and closed box during litter changing and feed warehouse has the highest fungal load in surfaces samples (Fig. 1). *Penicillium* sp. presented number of isolates impossible to count in air in closed box when brushing a horse. *Rhizopus* sp. also presented number of isolates impossible to count in air and surface samples in closed box during litter changing.

Besides the sampling site with higher air fungal load, also feed warehouse and closed box when brushing a horse presented more $CFU \cdot m^{-3}$ than the outdoor sample.

Eight different species of filamentous fungi were identified in air samples with a total of 260 isolates. *Aspergillus* genus presents the highest prevalence (53.8%). Species belonging to the *A. niger* complex (23.1%) and *A. fumigatus* complex (23.1%) were the most prevalent fungi in air sampling, but *A. flavus* complex was also isolated. *Syncephalastrum racemosum*, *Mucor* sp., *Penicillium* sp., *Scopulariopsis*

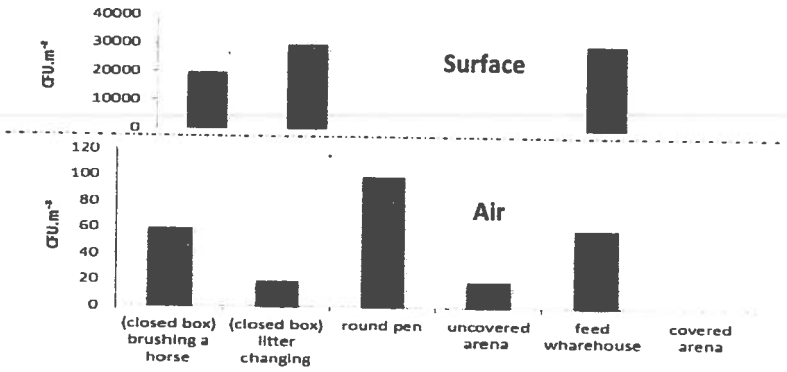

Figure 1. Fungal load distribution in the different sampling sites.

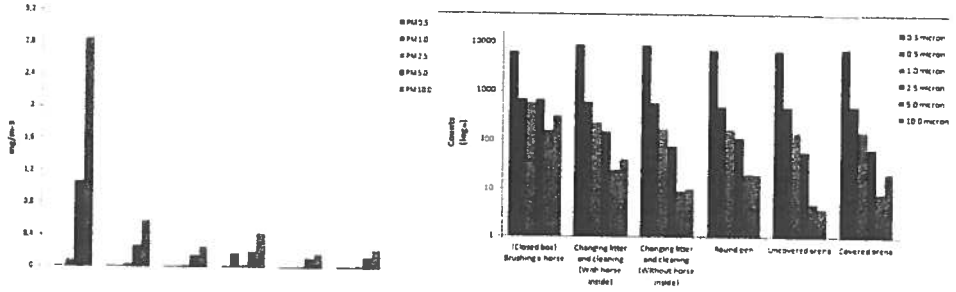

Figure 2. Distribution by size of mass (mg/m³) and particle number (counts).

brevicaulis and *Fusarium oxysporum* were also identified in the air samples.

Four different species were isolated in surfaces samples with a total of 80000 isolates. *Aspergillus* genus also presents the highest prevalence (87.5%). *Aspergillus niger* complex (375%) and *A. fumigatus* complex (37.5%) were also the most found, but *A. versicolor* complex was also isolated.

3.2 *Particles*

Were detected differences statistically significant for all particles sizes between tasks (p's < 0.0001). However, it appears that the PM_5 and PM_{10} present higher values in all the tasks studied. The higher median value for PM_{10} and PM_5 was obtained in the closed box during the horse brushing task (p < 0.000) (Fig. 2).

As regards the particle number concentration distribution by size, we detected statistically significant differences for all particle sizes between tasks (p = 0.000). Note that, the particle number concentration for 0.3 µm size presented higher number of particles in all the tasks with higher median value obtained in the closed box during the litter changing without the horse inside the box. Moreover, the tasks developed inside the closed box had the higher median values for almost all the sizes of particles (p's < 0.000) (Fig. 2).

4 DISCUSSION

Fungal contamination may contribute to an increase in allergies and many other adverse health effects in horse-stable workers. Fungi may be responsible for a variety of diseases: allergic reactions such as asthma, allergic rhinitis, hypersensitivity pneumonitis, infections caused by the growth of the fungus in body tissues and toxic reactions, mainly related to mycotoxins and fungal cell wall components (Górny et al., 2002).

Regarding to the health risks derived from exposure to fungi, the World Health Organization (WHO) considers the value of 150 $CFU \cdot m^{-3}$ as a reason for concern, especially when potentially pathogenic species of fungi are present (Goyer et al., 2001) as the ones most prevalent in our study belonging to *Aspergillus* genera. None of the sampling sites surpass the WHO value, however,

3 from the 6 sampling sites presented higher fungal load than outdoor sample meaning that there are sources of indoor fungal contamination (Wouters et al., 2006). Besides the quantitative assessment, is crucial to analyze the fungal species present, since adverse health effects are depended on fungal species (Hoog et al., 2002). According to the American Industrial Hygiene Association (AIHA, 1996) in the Field Guide for the Determination of Biological Contaminants in Environmental Samples, the identification of the species *A. flavus* and *A. fumigatus*, both of them identified in this setting, requires implementation of corrective measures.

Is important to highlight that mycotoxins occur in occupational environments whenever fungi are present (Mayer et al., 2008), thus besides fungal burden caused by the most common fungal species found, some of these strains are also known for their toxigenic potential, including species from *Penicillium* genus, and species from *A. fumigatus*, *A. niger* and *A. flavus* complexes. Consequently, we must potentially consider simultaneous exposure to several mycotoxins (Thrane et al., 2004). This situation is more critical when we are facing settings prone in dust since mycotoxins can be transported to workers respiratory system through particles (Allermann et al., 2000; Viegas et al., 2013).

To our knowledge, this is the first study developed in horse stables that comprehensively assess exposure to particles and obtained, besides mass values, data regarding particle number concentration by size. Moreover, in others research work (Berndt et al., 2010), data is given by workplace or horse management system and not by task developed in each workplace as in the present research. Therefore, the data obtained gives more accurate information regarding exposure and, consequently, allows the identification of the more suitable preventive and protective measures. In this case interventions on the workplaces and tasks developed in the closed box can have substantial impact on worker's exposure and health effects related.

Similar to our results, Samadi and colleagues (2009) found that feeding and cleaning the stables were the tasks with higher exposure to inhalable dust. Earlier studies have shown also higher particle load during cleaning of boxes and daytime activity in the stable, compared with the time of day when the boxes are without activity (Rosenthal et al., 2006; Riihimäki et al., 2008). Some studies have shown also that type and hygienic quality of feed and bedding material have also a great impact on dust concentration. Additionally, other variables need to be reflected, namely: type of measurements and exposure metrics used, the type of ventilation, air humidity and work routines (Riihimäki et al., 2008). All these variables can influence results and, consequently, their interpretation. Additionally, the particles results can be higher in the winter since in previous research work (Riihimäki et al., 2008) found higher particle load during the winter than in the summer. Probably natural ventilation is less effective during the winter season.

Besides mass and particle number concentration there are other aspects that must be contemplated when considering the particles related health effects, such as: chemical proprieties (Almeida-Silva et al., 2014) and also the fact that particles may act as a carrier and a source of nutrients for fungi (Viegas et al., 2012; Raulf et al., 2014), bacteria (Halstensen et al., 2013) acting as airborne allergens (Raulf et al., 2014). Particles are also rich in endotoxins from the cell wall of gram-negative bacteria and, as mentioned before, are also associated with the exposure to mycotoxins produced by several fungi (Allermann et al., 2000; Viegas et al., 2013).

5 CONCLUSIONS

The results obtained in this study highlight the need of further and more detail research regarding occupational exposures. Despite the need of more detailed data, the results point out to the need for applying adequate preventive and protective measures.

REFERENCES

Allermann, L. & Poulsen, O.M. 2000. Inflammatory potential of dust from waste handling facilities measured as IL-8 Secretion from lung epithelial cells in vitro. *Annals of Occupational Hygiene* 44(4): 259–269.

Berndt, A., Derksen, F.J. & Robinson, N.E. 2010. Endotoxin concentrations within the breathing zone of horses are higher in stables than on pasture. *The Veterinary Journal* 183: 54–57.

Elfman, L., Riihimaki, M., Pringle, J. & Walinder, R. 2009. Influence oh horse stable environment on human airways. *Journal of Occupational Medicine and Toxicology* 4: 10.

Gallagher, L.M., Crane, J., Fitzharris, P. & Michael, N. 2007. Bates Occupational respiratory health of New Zealand horse trainers. *Int Arch Occup Environ Health* 80: 335–341.

Goyer, N., Lavoie, J., Lazure, L. & Marchand, G., 2001. Bioaerosols in the workplace: Evaluation, control and prevention guide. *Institut de Recherche en Santé et en Sécurité du Travail du Québec.*

Górny, R.L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M. & Grhishpun, S.A. 2002. Fungal fragments as indoor air biocontaminants. *Appl Environ Microbiol* 68: 3522–3531.

Mayer, S., Engelhart, S., Kolk, A. & Blome, H. 2008. The significance of mycotoxins in the framework of assessing workplace related risks. *Mycotoxin Res*; 24: 151–164.

Raulf, M., Buters, J., Chapman, M., Cecchi, L., de Blay, F., Doekes, G., Eduard, W., Heederik, D., Jeebhay, M.F., Kespohl, S., Krop, E., Moscato, G., Pala, G., Quirce, S., Sander, I., Schleunssen, V., Sigsgaard, T., Walusiak-Skorupa, J., Wiszniewska, M., Wouters, I.M. & Annesi-Maesano, I. 2014. Monitoring of occupational and environmental aeroallergens—EAACI Position Paper. *Allergy* 69(10): 1280–1299.

Riihimäki, M., Raine, A., Elfman, L. & Pringle, J. 2008. Markers of respiratory inflammation in horses in relation to seasonal changes in air quality in a conventional racing stable. *The Canadian Journal of Veterinary Research* 72: 432–439.

Samadi, S.S., Wouters, I.M., Houben, R., Jamshifard, A.R., Van Eerdenburg, F.F. & Heederik, D.J.J. 2009. Exposure to Inhalable Dust, Endotoxins, b(1/3)-Glucans, and Airborne Microorganisms in Horse Stables. *Ann. Occup. Hyg.* 53(6): 595–603.

Schmid, O., Möller, W., Semmler-Behnke, M., Ferron, G.A., Karg, E., Lipka, J., et al. 2009. Dosimetry and toxicology of inhaled ultrafine particles. *Biomarkers* 14(S1): 67–73.

Thrane, U., Adler, A., Clasen, P.E., Galvano, F., Langseth, W. & Lew, H. 2004. Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae* and *Fusarium sporotrichioides*. *International Journal of Food Microbiology* 95: 257–266.

Viegas, S., Veiga, L., Malta-Vacas, J., Sabino, R., Figueredo, P., Almeida, A., et al. 2012. Occupational exposure to aflatoxin (AFB1) in poultry production. *Journal of Toxicology and Environmental Health, Part A* 75: 1330–1340.

Viegas, S., Veiga, L., Veríssimo, C., Sabino, R., Figueredo, P., Almeida, A., Carolino, E. & Viegas, C. a 2013. Occupational exposure to aflatoxin B1 in swine production and possible contamination sources. *Journal of Toxicology and Environmental Health, Part A: Current Issues* 76(15): 944–951.

Viegas, S., Veiga, L., Verissimo, C., Sabino, R., Figueredo, P., C. Viegas, et àl. b 2013. Occupational exposure to aflatoxin B1: the case of poultry and swine production. *World Mycotoxin Journal* 6(3): 309–315.

Viegas, S., Almeida-Silva, M. & Viegas, C. (forthcoming 2014). Occupational exposure to particulate matter in two Portuguese waste sorting units. International Journal of Occupational Medicine and Environmental Health.

Wouters, I., Spaan, S., Douwes, J.; Doekes, G. & Heederik, D. 2006. Overview of personal occupational exposure levels to inhalable dust, endotoxin, beta (1-->3)-glucan and fungal extracellular polysaccharides in the waste management chain. *Ann Occup Hyg.;* 50(1): 39–53.

Wichmann, H.E., Spix, C., Tuch, T., Wolke, G., Peters, A., Heinrich, J., et al. 2000. Daily mortality and fine and ultrafine particles in Erfurt, Germany. Part I: role or particle number and particle mass. Research Report 98, *Health Effects Institute, Cambridge, MA*.