

Identification and seasonal distribution of airborne fungi in three horse stables in Italy

Simona Nardoni¹, Francesca Mancianti¹, Micaela Sgorbini², Fabrizio Taccini³ & Michele Corazza²

¹Dipartimento di Patologia Animale, Profilassi ed Igiene degli Alimenti, Viale delle Piagge 2, 56124 Pisa, Italy; ²Dipartimento di Clinica Veterinaria, Università di Pisa, Italy; ³Dipartimento di Produzioni Animali, Università di Pisa, Italy

Received 14 October 2003; accepted in revised form 17 February 2004

Abstract

Fungal agents are responsible for a variety of respiratory diseases both in humans and animals. The nature and seasonal variations of fungi have been investigated in many environments with wide ranging results. The aims of the present report were (i) to evaluate the quality and magnitude of exposure to airborne fungi in three differently structured equine stalls (open air, partially and completely enclosed buildings) during a one-year period, using an air sampling technique and (ii) to compare the distribution and frequency of fungal species, with regards to these different environments. Air samples were collected monthly from December 2001 to November 2002 by means of a surface air sampler (SAS) Super-90, (PBI International, Milan, Italy). *Penicillium* and *Aspergillus* spp. were cultured from all the stables in all seasons. Mucoraceae were also recovered in all seasons in stalls 1 and 2, while they were not isolated in spring and fall in stall 3. These fungi were detected in 28.4%, 72.9% and 60.5% of the total number of samples, respectively. Other fungal genera such as *Alternaria*, *Cladosporium*, *Fusarium*, *Beauveria* and *Drechslera* were also occasionally recovered. Viable fungal concentrations varied greatly, ranging from below the limit of detection to more than 3000 CFU/m³ for stables 1 and 2, and 1750 CFU/m³ for stable 3. The median fungal concentration was approximately 178 CFU/m³. Total fungal concentration appeared to be highest in summer, winter and spring, and lowest in the fall.

Key words: Air sampling, environment, fungi, horse-stables, seasonal prevalence

Introduction

Airborne dust concentration has a profound influence on the respiratory health of both human and low animals. 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. High environmental fungal burdens have been shown to be involved in the pathogenesis of chronic bronchitis/alveolitis (CB), also called chronic obstructive

pulmonary disease (COPD) in stabled horses, characterised by cough, nasal discharge, expiratory dyspnoea and reduced performance. This disease, affecting 12–50% of such animals [16], shows an increased prevalence when high levels of airborne particles are present. It is also associated with the severity and duration of lower respiratory tract symptoms and diseases. Clinical signs usually regress when horses are removed from enclosed areas. Keeping them on pasture, removal of hay and straw are important to obtain symptom remission.

Fungal contamination may also 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 [8].

Environmental and seasonal variation of fungal aerosols have been investigated in building and outdoor environments such as schools, offices, residences [18], swine farms [6] in order to evaluate their role in causing adverse health effects. Moreover, a wide variety of matrices have been studied to establish the presence and identity of these fungi: air [2], house dusts [15, 19], water [4], and the conjunctiva of mares [3].

Investigations of the presence of airborne dusts and aeroallergen concentrations in horse stables have been reported in the literature [7, 23]. Due to differences in building structures, the level of contamination in such buildings may be different in open air stables than partially or completely enclosed buildings. To our knowledge, however, no investigations of the seasonal variation and prevalence of airborne fungi in stable environments have been carried out.

The aims of our study were: (i) to evaluate the quality and magnitude of exposure to airborne fungi in three differently structured stalls (open air, partially and completely enclosed buildings) over a one-year period using a volumetric air sampling technique and (ii) to compare the distribution and frequency of fungal species with respect to different stable architectures.

Materials and methods

Horses

The animals included in this study were adults of both genders with ages ranging from 8 to 14 years (mean 10.6; $\sigma = 2.26$) utilized for horse-jumping events. All the animals were managed in the same way. The management system had been introduced at least 1 month before sampling, and it was kept constant during the entire period of the study. The horses were bedded on straw and manually

fed dusty hay twice a day (in the morning and in the evening), and fodder was given at noon. The stalls were cleaned routinely on a daily basis. No materials were stored inside the stables.

All of the animals were vaccinated against tetanus and flu (Duvaxyn ie-t plus, Solvay Italia) and dewormed on a regular basis. All animals were considered healthy following physical examinations: a history of chronic respiratory disease was never reported.

Stables

The stables included in the study were situated in central Italy (Tuscany), and were built as follows:

1. Individual boxes (4×4 m), with a window opening on a central walkway and a concrete floor. Only the two ends of the central passage were in communication with the outside.
2. Individual boxes (3×4 m), in direct communication with the outside and a paddock in which about 6 horses were kept.
3. Open paddocks (8×8 m), each harbouring 2–3 animals.

The soil of both paddocks and posts were not covered with grass. The visual observation of the stables with respect to microfungi did not yield any evidence of molds [13].

Bioaerosol sampling

All samples were collected monthly from December 2001 to November 2002. The seasons were defined as winter (December, January and February), spring (March, April and May), summer (June, July and August) and fall (September, October and November). Each barn was sampled during all four seasons for the duration of the study. A total of 218 samples were analyzed. All sampling procedures were carried out in the absence of wind. Environmental temperatures and relative humidity were recorded using direct-reading instruments.

A surface air sampler (SAS) Super-90, (PBI International, Milan, Italy) was employed. This volumetric sampler aspirates air at a fixed speed for variable periods of time through a perforated plate containing 219-holes positioned over a Petri plate containing agar growth medium. The

sampler was operated at a calibrated flow rate of 20 l/min, at an air inlet height of 1.5 m.

Air samples were cultured on 55 mm contact plates (Bibby Sterilin Ltd., Stone Staffs, UK), each filled with 16 ml of malt extract agar (MEA). The duration of sampling was 3 min. After sampling, the plates were covered, incubated at 25 °C and checked for growth on the 4th day of incubation, and then daily up to 15 days. Colonies of filamentous fungi were identified to the genus level on the basis of macro- and microscopic features. For *Aspergillus* isolates, identification was conducted to the species level following Raper and Fennell [14]. On the basis of the number of fungal colonies on each plate and the air volume sampled, the number of colony forming units per cubic meter of air (CFU/m³) was calculated.

Statistical analysis

Air samples from each stable were analyzed using descriptive statistics, such as median, mean, and 10%, 25%, 75% and 95% quantiles. The Wilcoxon/Kruskal–Wallis test was used to compare statistically significant differences between fungal presence and concentration by season and stable.

Results

The values of fungal concentrations showed great differences, ranging from below the limit of detection to more than 3000 CFU/m³ for stables 1 and 2, and 1750 CFU/m³ for stable 3, respectively. The median fungal concentration was approximately 178 CFU/m³. Means and standard deviations are reported in Table 1.

Mucoraceae, *Penicillium* and *Aspergillus* were cultured from all the stables. These groups detected in 28.4%, 72.9% and 60.5% of the total number of samples, respectively. In most cases, more than one fungal species was cultured from each plate. For fungi belonging to the genus *Aspergillus*, the following species were isolated: *A. niger* (31.6%), *A. flavus* (12.4%), *A. fumigatus* (8.7%) and *A. terreus* (7.8%). More detailed data are presented in Table 2. Species of the Mucoraceae were detected in all seasons in stalls 1 and 2. They were not isolated in spring and fall in stall 3. *Penicillium* species were detected in the three stalls in all seasons, while *A. niger* was present in all

Table 1. Concentrations of airborne fungi (expressed as CFU) measured in three types of stables in a one-year seasonal sampling

Fungi	Winter			Spring			Summer			Fall		
	Stable 1	Stable 2	Stable 3	Stable 1	Stable 2	Stable 3	Stable 1	Stable 2	Stable 3	Stable 1	Stable 2	Stable 3
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	Std.	Std.	Std.	Std.	Std.	Std.	Std.	Std.	Std.	Std.	Std.	Std.
	Dev.	Dev.	Dev.	Dev.	Dev.	Dev.	Dev.	Dev.	Dev.	Dev.	Dev.	Dev.
Mucoraceae	2333	1019,6442	271.5	355	63.6	123.5	89.8	915	1265.7	840	1001.5	1416.3
<i>Penicillium</i>	966.5	914.3	1186.5	1163.281	114.5	958.5	482.9	939	577	760	1854	2271.2
spp.												
<i>A. fumigatus</i>	10	18	25.4	86	121.6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
<i>A. niger</i>	56	38.2	224	288.5	495.5	0.7	57.5	81.3	1238	1671.6	10	14.1
<i>A. flavus</i>	10	14.1	43.5	23.3	196.5	< LOD	< LOD	20	28.3	< LOD	< LOD	< LOD
<i>A. terreus</i>	10	14.1	42.5	60.1	< LOD	< LOD	< LOD	10	14.1	< LOD	< LOD	< LOD

*Below the limit of detection.

Table 2. Percentages of stables positive for isolated fungal types by season

Fungi	Winter (%)			Spring (%)			Summer (%)			Fall (%)		
	Stable 1	Stable 2	Stable 3	Stable 1	Stable 2	Stable 3	Stable 1	Stable 2	Stable 3	Stable 1	Stable 2	Stable 3
Mucoraceae	71.4	80.6	52.9	92.3	72.2	66.7	37.5	40	71.4	100	93.3	100
<i>Penicillium</i> spp.	57.1	29	29.4	30.8	16.7	< LOD*	6.2	35	28.6	4	40	< LOD
<i>A. fumigatus</i>	2.4	16.1	29.4	7.7	< LOD	< LOD	12.5	10	< LOD	< LOD	< LOD	50
<i>A. niger</i>	40.5	22.6	82.3	30.8	50	66.7	25	15	14.3	12	20	< LOD
<i>A. flavus</i>	2.4	25.8	52.9	< LOD	5.5	< LOD	50	< LOD	< LOD	< LOD	< LOD	< LOD
<i>A. terreus</i>	< LOD	9.7	52.9	7.7	5.5	< LOD	6.2	10	< LOD	< LOD	< LOD	< LOD

*Below the limit of detection.

seasons in stalls 1 and 2. The latter was never recovered in the fall in stable 3.

When data concerning the three stables were collectively considered, total fungal concentrations appeared to vary by season ($p = 0.03$) being highest in summer, winter and spring, and lowest in the fall. No significant variations were observed evaluating the results obtained considering each stable individually. The airborne concentration of culturable fungi did not vary significantly according to stable architecture. No statistically significant differences were observed in the seasonal distribution of fungal species in the stables, except for the *A. niger* levels, which peaked in winter and spring ($p = 0.02$) in all the stables. Other fungal taxa such as *Alternaria*, *Cladosporium*, *Fusarium*, *Beauveria* and *Drechslera* were recovered occasionally in spring and summer only. In particular from stable 1, 350 CFU/m³ of *Cladosporium* were isolated in spring and 500 CFU/m³ of *Alternaria* were obtained in summer. One hundred CFU/m³ of *Scopulariopsis* sp. were isolated from stable 2 in summer and 1300 CFU/m³ of *Cladosporium* sp., 500 of *Drechslera* sp., 250 of *Beauveria* sp. and 1900 of *Fusarium* sp. were cultured from air in stable 3 in summer.

Discussion

Published data on the presence and prevalence of fungi in stables are limited. The seasonal prevalence of viable molds with particular reference to *Aspergillus* has been studied in urban areas by several investigators with varied results. The seasonal effects on the presence and quantity of airborne fungi were investigated in air and lake water

in Turkey [4], and houses in Taiwan [19] and Saudi Arabia [2]. No significant differences in fungal prevalence were observed in urban areas of the United States [15]. The fungal concentrations found in our study showed a seasonal variation that was not comparable to surveillance results obtained by other investigators from similar environments. The air samples obtained by Ferro et al. [7] in stables were collected to achieve a qualitative evaluation of environmental dust, so a comparative evaluation with our data could not be carried out. However, *Aspergillus* and *Penicillium* species, most frequently encountered by these investigators from samples of air, bedding and hay of Northern Italian stables, were also identified in the present study.

SAS was employed by us for sampling viable fungi: it is only 50% efficient for particles of the size of the *Aspergillus* conidia, but it offers significant advantages in terms of practicality [12], and it has been shown to be sufficiently efficient to perform *Cladosporium* air counts [5]. Therefore the low rate of recovery of such fungi in the present work should not be considered an underestimate.

Fungal concentrations have been widely investigated in barns housing other animal species, especially pigs. *Aspergillus* and *Penicillium* spp. were also detected in minor amounts [6]. The conidia of these fungi have been regarded as common aeroallergens, in part because their size range is between 2–4 μ m in diameter: thus, they belong to the respirable fraction (RF), which is able to reach the peripheral airways. Conidia of *Aspergillus* spp. may aggregate into clumps of chains with different aerodynamic properties with implications for particle capture instruments [10, 12]; however, Gorny et al. [8] reported that

Aspergilli and *Penicilli* do occur in the air as single spores. A survey on the conidial size distribution in bioaerosols should employ volumetric spore traps as reported by Hasnain et al. [9] and would be of interest for further investigations.

The presence of high concentrations of potentially pathogenic airborne fungi may pose health risks to susceptible humans and animals.

In the present study, airborne culturable fungal levels showed slight differences in the three types of stables. It can be concluded that, in this case, the building's structure seemed not to influence the presence of airborne fungi, even if taxa other than *Aspergillus*, *Penicillium* and Mucoraceae were mostly cultured from stable 3, a structure that was more open to the outside, particularly in spring. Consequently, the influence of the management system in the determination of airborne fungal concentrations may be important, as suggested by Woods et al. [23] who demonstrated the importance of low dust-producing materials such as wood shaving bedding and a complete pelleted diet as a means to decrease the respirable dust burden in horse stables.

The results of air sampling for viable fungi are considered to be highly dependent on the method used. For this reason, our air sampling was highly standardized, and identical methods were followed in collecting all samples. All analyses were conducted in duplicate in the same laboratory to confirm the results. It is known that culture methods underestimate the concentration of airborne microorganisms present in the environment. Culture, however, is still one of the most popular methods utilized in bioaerosol field studies, permitting quantification as well as determination to genus and species level [6].

The sources of fungal bioaerosol in sampled environments cannot easily be determined due to ubiquitous nature of the taxa. *Penicillia* are ubiquitous, frequently found in soil and on decomposing organic debris; Mucoraceae are widely distributed on stored grain, fruits, vegetables and in compost. *Aspergillus fumigatus* is a thermotolerant species frequently reported as a predominant mold in moist plant materials undergoing rapid decomposition, often with heating. *Aspergillus flavus* like the black aspergilli, such as *A. niger*, is encountered more frequently than any other group in the genus. They occur in and upon the greatest variety of substrata, including grains, forage products, protein rich

substrata and decaying vegetation in warm climates. In contrast, *A. terreus* are typically soil dwelling [14]. *Aspergilli* have been reported as causative agents of allergic and invasive disease both in humans and animals. Inhalation of *A. fumigatus* conidia have been reported to induce pulmonary disease in horses affected with COPD, implicating this fungal species in the etiology of the disease [11, 17]. This species was also described as the etiological agent of mycotic keratitis in a horse in Finland [1]. Other species such as *A. flavus* are implicated in the pathogenesis of respiratory infections. *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *A. terreus* are also reported to produce mycotoxins that can cause equine deaths [20, 22]. *Aspergillus terreus* is considered an important emerging fungal pathogen that is resistant to amphotericin B both *in vitro* and in experimentally induced pulmonary infections [21].

A good management system designed to minimize the risks of allergic respiratory diseases should consider such factors as: (i) the presence of grass instead of exposed soil both in posts and paddocks, (ii) the utilization of wood shavings for bedding and (iii) the importance of good quality hay for feeding animals, and trying to decrease the degree of agitation of such materials [23].

References

1. Aho R, Tala M, Kivalo M. Mycotic keratitis in a horse caused by *Aspergillus fumigatus* The first reported case in Finland. Acta Vet Scand 1991; 32: 373–376.
2. Al-Suwaine AS, Bahkali AH, Hasnain SM. Seasonal incidence of airborne fungal allergens in Riyadh, Saudi Arabia. Mycopathologia 1999; 145: 15–22.
3. Andrew SE, Nguyen A, Jones GL, Brooks DE. Seasonal effects on the aerobic bacterial and fungal conjunctival flora of normal thoroughbred brood mares in Florida. Vet Ophthalmol 2003; 6: 45–50.
4. Asan A, Kirgiz T, Sen B, Camur-Elipek B, Guner U, Guher H. Isolation, identification and seasonal distribution of airborne and waterborne fungi in Terkos Lake (Istanbul, Turkey). J. Basic Microbiol 2003; 43: 83–95.
5. Bellin P, Schillinger J. Comparison of field performance of the Andersen N6 single stage and the SAS sampler for airborne fungal propagules. Indoor Air 2001; 11: 65–68.
6. Chang CW, Chung H, Huang CF, Su HJJ. Exposure of workers to airborne micro organisms in open-air swine houses. Appl Environ Microbiol 2001; 67: 155–161.
7. Ferro E, Ferrucci F, Salimei E, Antonini M, Codazza D, Caniatti M. Relationship between the conditions of lower airways in healthy horses, environmental factors and air quality in stables. Pferdeheilkunde 2000; 16: 579–586.

8. Górny RL, Reponen T, Willeke K, Schmechel D, Robine E, Boissier M, Grishpun SA. Fungal fragments as indoor air biocontaminants. *Appl Environ Microbiol* 2002; 68: 3522–3531.
9. Hasnain SM, Al-Frayh AS, Al-Suwaine A, Gad-El-Rab MO, Fatima Al-Sedairy S. *Cladosporium* and respiratory allergy: diagnostic implications in Saudi Arabia. *Mycopathologia* 2004; 157: 171–179.
10. Lacey J. Aggregation of spores and its effects on aerodynamic behaviour. *Grana* 1991; 30: 437–445.
11. McGorum BC, Dixon PM, Halliwell RE. Responses of horses affected with chronic obstructive pulmonary disease to inhalation challenges with mould antigens. *Equine Vet J* 1993; 25: 257–258.
12. Morris G, Kokki MH, Anderson K, Richardson MD. Sampling of *Aspergillus* spores in air. *J. Hosp Infect* 2000; 44: 81–92.
13. Portnoy JM, Barnes CS, Kennedy K. Sampling for indoor fungi. *J. Allergy Clin Immunol* 2004; 113: 189–198.
14. Raper KB, Fennell DI. The genus *Aspergillus*. ed. Baltimore: The Williams and Wilkins Company, 1965.
15. Ren P, Jankun TM, Leaderer BP. Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American country. *J. Expo Anal Environ Epidemiol* 1999; 9: 560–568.
16. Robinson NE, Derksen FJ, Olzewski MA, Buechner-Maxwell VA. The pathogenesis of chronic obstructive pulmonary disease of horses. *Br Vet J* 1996; 152: 283–306.
17. Schmallenbach KH, Rahman I, Sasse HHL, Dixon PM, Halliwell REW, McGorum BB, Crameri R, Miller HRP. Studies on pulmonary and systemic *Aspergillus fumigatus*-specific IgE and IgG antibodies in horses affected with chronic obstructive pulmonary disease (COPD). *Vet Immunol Immunopathol* 1998; 66: 245–256.
18. Shelton BG, Kirkland KH, Flanders WD, Morris G. Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl Env Microbiol* 2002; 68: 1743–1753.
19. Su HJ, Wu PC, Chen HL, Lee FC, Lin LL. Exposure assessment of indoor allergens, endotoxin, and airborne fungi for homes in southern Taiwan. *Environ Res* 2001; 85: 135–144.
20. Vesonder R, Haliburton J, Stubblefield R, Gilmore W, Peterson S. *Aspergillus flavus* and aflatoxins B1, B2, and M1 in corn associated with equine death. *Arch Environ Contam Toxicol* 1991; 20: 151–153.
21. Walsh TJ, Petraitis V, Petraitiene R. Experimental pulmonary aspergillosis due to *Aspergillus terreus*: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. *J. Infect Dis* 2003; 15(188): 305–319.
22. Wilson DM, Mubatanhema W, Jurjevic Z. Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. *Adv Exp Med Biol* 2002; 504: 3–17.
23. Woods PSA, Robinson NE, Swanson MC. Airborne dust and aeroallergen concentration in a horse stable under two different management systems. *Equine Vet J* 1993; 25: 208–213.

Address for correspondence: Dr. Simona Nardoni, Dipartimento di Patologia Animale, Profilassi ed Igiene degli Alimenti, Viale delle Piagge 2, 56124 Pisa, Italy
 Phone: + 39(0)50570032; Fax: + 39(0)50540644
 E-mail: snardoni@vet.unipi.it