SAMPLING AND DIFFERENTIATION OF AIRBORNE MOLDS IN ANIMAL HOUSES

Wolfgang Müller, Uta Schütze, Jochen Schulz and Bert-Andree Zucker
Institut für Tier- und Umwelthygiene, Freie Universität Berlin, Berlin, Germany

Introduction

Airborne molds in animal houses may affect the health of animals and working personal in many ways (Singh, 2004). They can cause a number of different types of illness, through the production of spores, mycotoxins and VOC emissions, for example:

- Allergic reactions (rhinitis and asthma)
- Hypersensitivity pneumonitis
- Infection, for example Aspergillosis
- Inflammation from fungal cell wall components
- Immune disorder and cancer from mycotoxins
- Irritation from fungal VOCs.

In this study the concentration and the genus composition of airborne molds was determined in different animal houses. Further the inflammation inducing potential of molds was characterized.

Material and Methods

In pilot experiments different aerosol samplers [KROTOW slit-sampler, 6-stage ANDERSEN cascade-impactor, REUTER centrifugal air sampler (RCS, firm Biotest GmbH), all-glass-impinger (AGI-30)] and culture media (SABOURAUD-Glucose-nutrient agar, Bierwürze-Pepton-nutrient agar, Bengalrot-Chloramphenicol-nutrient agar, Dichloran-Glycerin-nutrient agar) were tested for their ability to collect airborne molds in animal houses.

Based on the results of the pilot experiments the concentration of airborne molds were studied in cattle, sheep, pig and poultry houses. Grown molds were stained with cotton blue and identified microscopically using morphological characters.

The inflammation inducing potential was characterized from mold species that were frequently isolated from the airborne state of animals houses by using human whole blood cytokine response. The test procedure is described in detail elsewhere (Zucker, 2004). Briefly, samples of interest are incubated with diluted blood from healthy human donors. After contact with relevant structures monocytes release proinflammatory signal molecules such as interleucin-1ß (IL-1ß). IL-1ß release is quantified by ELISA measurement.

Results and Discussion

- Pilot experiment

The highest collection efficiency was achieved with the Andersen sampler and the Dichloran-Glycerin-agar. Therefore, the Andersen sampler and the Dichloran-Glycerin-agar were used for all further investigations.

The sampler was operated at an airflow of 28.3 l/min 1 m above the ground in the center of the animal houses. The concentrations of microorganisms in 1 m³ of air were calculated from the colony count and airflow, and expressed as cfu/m³. Further the Andersen sampler was used to determine the aerodynamic size of the isolated molds.

- Concentration and genus composition

On average the total amount of airborne molds ranged from 1,8 x 10^2 to 7,5 x 10^3 cfu/m³ in cattle barns, from 2,3 x10^2 to 5,6 x 10^3 cfu/m³ in pig houses and from 7,2 x 10^2 cfu/m³ to 1,2 x 10^5 cfu/m³ in poultry houses. That concentration are comparable to those reported from other investigations in animal houses (e.g. Gemeinhardt und Wallenstein, 1985; Cormier et al., 1990).

Aspergillus, *Penicillium*, *Cladosporium* and *Scopulariopsis* were the most frequently identified mold genera followed by the genera *Alternaria* and *Wallemia* (Table 1). Some genera isolated in this study are known to induce allergic reactions in humans and animals. However to access the "allergic potential" of an bioaerosol the concentration of the specific allergens should be determined. Therefore it is of interest how the number of culturable molds correlates with the concentration of specific allergens, e.g. the number of airborne *Aspergillus fumigatus* and the concentration of Asp f 1 or the number of *Alternaria alternata* and the concentration of Alt a 1.

Table 1: Number of investigated air-samples positive for different genera of molds

Stable	n	*Pen.*	*Asp.*	*Sco.*	*Cla.*	*Alt.*	*Wal.*
Cattle	37 (100%)	30 (81%)	35 (95%)	14 (38%)	36 (97%)	20 (54%)	13 (35%)
Pig	22 (100%)	20 (91%)	22 (100%)	20 (91%)	20 (91%)	13 (59%)	8 (36%)
Poul-try	20 (100%)	19 (95%)	19 (95%)	15 (75%)	12 (60%)	10 (50%)	13 (65%)
Sheep	26 (100%)	25 (96%)	25 (96%)	20 (77%)	15 (58%)	6 (23%)	26 (100%)

n = number of investigated samples, *Pen. = Penicillium, Asp. = Aspergillus, Cla. = Cladosporium, Sco. = Scopulariopsis, Alt. = Alternaria, Wal. = Wallemia*

- Aerodynamic sizes

Most of airborne *Aspergillus*, *Penicillium*, *Cladosporium*, *Scopulariopsis* and *Wallemia* are able to penetrate into the lungs, and a considerable part of *Aspergillus*, *Penicillium* and *Wallemia* can even penetrate into the alveoli. In table 2 the percentage of airborne molds that are able to penetrate into the lung and into the alveoli is shown.

Table 2: Percentage of airborne molds found on stage 6 and stage 3-6 of the Andersen sampler

Stable	Stage of the Andersen sampler 3-6	6
Cattle	71,4%	2,9%
Pig	69,3%	2,6%
Poultry	52,0%	5,3%
Sheep	77,7%	5,1%

Stage 3-6 penetration into the lung, Stage 6 penetration into the alveoli

- Inflammation-inducing potential

In table 3 the minimal concentrations of different microorganisms as well as lipopolysaccharides (cell wall component of gram-negative bacteria) and ß-Glucans (cell wall component of gram-positive bacteria and molds) are shown that are necessary to induce IL-1ß in the human whole blood assay. The potency of molds to induce inflammation is similar to that of gram-positive bacteria, but clearly less than that of gram-negative bacteria. However, it should considered that bioaerosols from animal houses almost always contain different microbial components. Since lipopolysaccharides and glucans activate macrophages/monocytes via different pathways (Takeuchi and Akira, 2001) it is possible that both substances exhibit synergistic effects after simultaneously stimulation.

Table 3: Minimal concentration of different microorganisms and cell wall components of microorganisms that are necessary to induce IL-1ß in the human whole blood assay

Microorganism	Concentration
Aspergillus fumigatus	$3{,}3 \times 10^6$ cfu/ml
Penicillium puberulum	$1{,}6 \times 10^6$ cfu/ml
E. coli D12/4/99	$8{,}8 \times 10^2$ cfu/ml
Staphylococcus xylosus	$1{,}1 \times 10^6$ cfu/ml
E. coli-LPS (O111:B4)	10 ng/ml
Zymosan	100 µg/ml
Laminarin	1 mg/ml
Curdlan	1 mg/ml

Conclusions

In the air of animal houses regularly mould genera/species can be found which might lead to allergic diseases. Furthermore cell wall component of fungi are able to induce inflammatory reactions after inhalation. However, their inflammation-inducing potential is low compared to endotoxins.

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