Spore exposure arising from stored hay, grain and straw

MARJUT KOTIMAA

Kuopio Regional Institute of Occupational Health, P.O.B. 93, SF-70701 Kuopio, Finland

Abstract. The quantitative and qualitative differences in microbe exposure arising from hay, grain and straw during the end of the indoor feeding period were investigated by using a six-stage fractionating impactor (model 10—800, Andersen Inc.). Straw samples (n = 5) liberated significantly higher amounts of spores \(3.7 \times 10^6 \text{ cfu/m}^3\) in comparison to hay samples (n = 33) and grain samples (n = 2), which liberated \(0.6 \times 10^6 \text{ cfu/m}^3\) and \(0.2 \times 10^6 \text{ cfu/m}^3\), respectively. Thermotolerant and thermophilic microflora were typical of the exposure originating from straw. Hay liberated about 10% and grain only 0.7%, the level of spores of thermotolerant fungi liberated from straw. The corresponding percentages of spores of thermophilic actinomycetes were 5% and 0.4%. Thermoauctinomyces vulgaris was the dominating microbe in the exposure caused by straw; Aspergillus umbrosus was the major species in the microflora liberated from hay and grain. Other Aspergillus (A.) species (A. fumigatus, A. ochraceus, A. flavus, A. repens, A. versicolor) and Penicillium (P.) species (P. expansum, P. piceum, P. citrinum, P. brevicompactum, P. echinulatum, P. verrucosum var. cyclopium) occurred frequently, and in great amounts, in all the analysed materials. Spores of Cladosporium (C.) species (mainly C. herbarum, C. cladosporioides, and C. macrocarpum) were found frequently, and abundantly, during the handling of hay. The present results suggest that not only the traditional causative agents of farmer’s lung disease but also other fungal and actinomycete species may be found in high concentrations during the handling of bedding and feeding stuffs, and that these fungal and actinomycete exposures may cause respiratory symptoms and other health problems in both man and animals. Special attention should be paid to decreasing the moisture content of hay and straw before storing in order to lower the risk of moulding during the indoor feeding period.

Introduction

Dust problems are typical of agricultural working environments. Dust exposure consists mostly of organic particles, which originate from feeding and bedding stuffs and from animals and their excrements. Organic components may include, e.g. animal dander, hair, feathers, manure, insects, mites, pollen, fungal spores or fragments of fungal hypha, bacteria and their endotoxins, mycotoxins and fodder particles. The amount and the quality of dust are affected by the branch of production, geographical location and the climatic conditions, and these factors are also related to the prevalence and the incidence of farm-
er's lung (Terho et al. 1987, Vohlonen et al. 1987). Farmer's lung disease is one type of allergic alveolitis caused by fungal and actinomycete spores arising from mouldy plant material (Pepys 1969).

The yearly incidence of allergic alveolitis in Finland has increased steadily from 101 cases in 1984 to 340 cases in 1988. The vast majority of the cases occurs among farmers (Vaaranen et al. 1985, 1986, 1987, 1988, 1989), especially on dairy farms. Disease similar to farmer's lung has also been reported in bovines and in horses (Pirie et al. 1971, Wise-man et al. 1973, Asmundsson et al. 1983). Hay has been accused of causing the disease, although other stored plant materials (feeding and bedding stuffs) used on farms are as susceptible to moulding as hay. The aim of the present study was to investigate quantitative and qualitative differences in microbe exposure arising from hay, grain and straw during the end of the indoor feeding period.

Material and methods

Material samples for aerobiological studies were taken at the end of indoor feeding period (in April and May) on the farms, which situated in Eastern Finland. Thirty-three farms were included in the study and hay samples were taken from baled hay, grain samples from grain which had been dried with unheated forced air and straw samples from baled straw. All the material samples represented average quality, exceptionally mouldy or good quality batches were excluded.

A six-stage fractionating impactor (model 10-800, Andersen Inc., Georgia, USA) was used to take air samples for analysing the quality and the quantity of viable microflora (Andersen 1958). Samples were taken during the handling of hay (N = 33), grain (N = 2) and straw (N = 5), at a distance of half a metre from the farmer's breathing zone.

Each sample included four successive measurements. Two sets of Hagen-medium (malt extract-glucose-agar (Russel 1974) supplemented by 35 mg streptomycin and 35 mg Rose Bengal and diluted to 1000 ml medium) were used. One sample was incubated at 20 °C for the outgrowth of mesophilic fungi and the other was incubated at 40 °C to obtain colonies of thermotolerant fungi. NaCl-malt extract agar (Terho 1978) (incubation at 20 °C) was used for Aspergillus (A.) glaucus group fungi, and half-strength Nutrient agar (Corbaz et al. 1963) (incubation at 55 °C) for thermophilic actinomycetes. After incubation, the colonies were identified using a light microscope and counted. The positive hole correction method of Andersen (1958) was used to count colonies before calculating the concentrations, which are expressed as colony-forming units per cubic metre of air (cfu/m³). The sampling time per medium varied from 5 to 30 seconds, according to the visible mouldiness of the material. In evaluating the differences in spore concentrations, one-way analysis of variance was used after the logarithmic transformation of calculated values. The Chi square test was applied to evaluate the differences in the frequencies of various microbes.

Results

Handling of feeding and bedding stuffs caused a high level of exposure, from 10⁴ to 10⁷ cfu/m³. Both the lowest and the highest total spore value was measured during the handling of hay (19 000 cfu/m³ and 13 700 000 cfu/m³, respectively) (Table 1). In all cases, straw liberated large amounts of spores, the difference between straw and other materials being statistically significant (F = 3.40, p < 0.05). Compared to hay and straw, grain samples caused only slight exposure to spores. Thermotolerant and thermophilic microflora were typical of the exposure originating from straw. Hay liberated about 10 % and grain only 0.7 %, the level of spores of thermotolerant fungi liberated from straw. The corresponding percentages of
Table 1. The concentration of airborne spores of different microbe groups expressed as geometric means (x) of colony forming units per m$^3$ during the handling of various materials on farms.

| Microbe group                        | Hay $\times 10^5$ | Straw $\times 10^5$ | Grain $\times 10^5$ |
|--------------------------------------|-------------------|---------------------|---------------------|
| Mesophilic fungi range               | 380               | 1900                | 160                 |
|                                      | (9.7—6600)        | (520—6500)          | (38—650)            |
| Thermotolerant fungi range           | 24                | 230                 | 1.6                 |
|                                      | (0.05—2000)       | (12—2600)           | (0.25—11)           |
| Thermophilic actinomycetes range     | 36                | 670                 | 1.6                 |
|                                      | (0.07—5100)       | (100—3300)          | (0.39—11)           |
| Total                                | 630               | 3700                | 160                 |
| range                                | (19—14000)        | (2100—12000)        | (38—670)            |

Spores of thermophilic actinomycetes were 5% and 0.4% (F=3.79, p<0.05). *Thermoactinomyces vulgaris* was the dominating microbe in the exposure caused by straw (F=3.61, p<0.001); *Aspergillus umbrosus* was the major species in the microflora liberated from hay and grain (Table 2). Other *Aspergillus* species (*A. fumigatus, A. ochraceus, A. flavus, A. repens, A. versicolor*) and *Penicillium* species (*P. expansum, P. piceum, P. citrinum, P. brevicompactum, P. echinulatum, P. verrucosum var. cyclopium*) occurred frequently and in great amounts in all the analysed materials. Of the fungi that were found occasionally, or in minor concentrations, *Humicola* sp. was significantly more common in straw than in hay or grain (F = 3.93, p<0.05, X² = 4.75, p<0.10) (Table 2). The spores of the *Cladosporium* species (mainly *C. herbarum, C. cladosporioides* and *C. macrocarpum*) were found frequently, and abundantly, during the handling of hay (F = 4.35, p<0.05, X² = 6.12, p<0.05).

Discussion

Each year, most cases of farmer’s lung are diagnosed during the end of the indoor feeding period (Terho et al. 1980, Pether & Greatorex 1976). During that time the exposure to airborne spores is greater than at the beginning of the indoor feeding period and small-spored storage fungi are mainly encoun-tered (Kotimaa et al. 1978, 1981). A similar incidence pattern has also been reported among bovines, which contract respiratory disorders after having been fed mouldy hay (Pirie et al. 1971, Wiseman et al. 1973). All samples were collected for this study during the season involving the highest exposure to spores, though there were noticeable differences in the quality and the quantity of exposure to spores during the handling of different materials.

Straw bedding caused the highest spore concentrations when compared to hay or grain; parallel results have also been published in other reports (Mulinge & Chester 1970, Lacey 1971). The role of straw as a factor increasing exposure to spores on farms has not received much attention so far. The great numbers of thermotolerant fungi and thermophilic actinomycetes indicate spontaneous heating resulting from the high moisture content of stored material (Festenstein et al. 1965). Straw is collected, often by baling, when the weather is often rainy, or at least when the difference in temperature between the daytime and the night-time is great, and thus dew may provide sufficient moisture to initiate moulding. It has not been studied how straw could be collected and preserved without giving rise to conditions favourable to moulding. The quality of the microbe exposure originating from straw was much the same as that originating from hay and causing
Table 2. Concentration of the spores of different taxons, expressed as geometric means (x) of colony forming units per m³, and their prevalence (%) during the handling of stored hay, straw and grain.

| Taxon                      | Hay   |          |          |          | Straw  |          |          |          | Grain  |          |          |
|----------------------------|-------|----------|----------|----------|--------|----------|----------|----------|--------|----------|----------|
|                            | x     | Range    | %        | x        | Range  | %        | x        | Range    | %      | x        | Range    |
| Alternaria spp             | 8     | 0—12 000 | 30.3     | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Aspergillus spp            | 79    | 0—230 000| 48.5     | 580      | 600—630 000 | 60.0     | 6        | 0—36     | 50.0   | 1 100    | 220—5 400 | 100.0   |
| A. fumigatus               | 11 000| 48—2 000 000 | 100.0     | 580      | 1 000—1 100 000 | 60.0     | 1 100    | 220—5 400 | 100.0  | 0        | —        |
| A. niger                   | 12    | 0—1 400 000 | 30.3     | 7        | 0—22 000 | 20.0     | 0        | —        | 0.0    | 0        | —        |
| A. umbrosus                | 76 000| 1 000—5 900 000 | 100.0     | 50 000   | 1 700—5 200 000 | 100.0    | 84 000   | 37 000—190 000 | 100.0 |
| Aur. pullulans             | 2     | 0—430    | 9.0      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Botryotrichum sp           | 0     | —        | 0.0      | 4        | 0—860  | 20.0     | 0        | —        | 0.0    | 0        | —        |
| B. cinerea                 | 2     | 0—1 000  | 15.2     | 15       | 0—1 700 | 40.0     | 0        | —        | 0.0    | 0        | —        |
| Candida sp                 | 2     | 0—2 400  | 6.1      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Chaetomium sp              | 1     | 0—36     | 3.0      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Cladosporium spp           | 850   | 0—140 000| 78.8     | 26       | 0—7 100 | 40.0     | 0        | —        | 0.0    | 0        | —        |
| Gloeophyllum sp            | 1     | 0—18 000 | 3.0      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Haplographium sp           | 1     | 0—1 500  | 3.0      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Humicola spp               | 4     | 0—93 000 | 19.2     | 480      | 2 000—600 000 | 60.0     | 15       | 0—210    | 50.0   | 150      | 110—210  | 100.0   |
| M. faeni                   | 61    | 0—260 000| 48.5     | 550      | 0—110 000 | 40.0     | 150      | 110—210  | 100.0  | 29       | 0—860    | 50.0    |
| Mucor spp                  | 1 900 | 0—720 000| 90.9     | 870      | 0—43 000 | 80.0     | 46       | 0—2 100  | 50.0   | 46       | 0—2 100  | 50.0    |
| P. variotii                | 16    | 0—5 300   | 42.4     | 14       | 0—2 600  | 40.0     | 15       | 0—210    | 50.0   | 15       | 0—210    | 50.0    |
| Penicillium spp            | 34 000| 0—2 300 000| 93.9     | 20 000   | 2 900—7 900 000 | 100.0    | 7 000    | 110—460 000 | 100.0 |
| Rhizopus spp               | 7     | 0—2 500  | 33.3     | 45       | 0—1 500 | 60.0     | 0        | —        | 0.0    | 0        | —        |
| S. brevicaulis             | 2     | 0—130 000| 12.1     | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Sporabolomyces sp          | 2     | 0—2 700  | 6.1      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Streptomyces spp           | 86    | 0—42 000 | 60.6     | 37       | 0—21 000 | 40.0     | 15       | 0—210    | 50.0   | 15       | 0—210    | 50.0    |
| T. sacchari                | 1     | 0—130    | 3.0      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| T. vulgaris                | 19 000| 48—5 000 000 | 100.0     | 560 000  | 9 400—3 000 000 | 100.0    | 1 100    | 110—11 000 | 100.0  | 0        | —        |
| Th. viridis                | 0     | —        | 0.0      | 12       | 0—210 000 | 20.0     | 0        | —        | 0.0    | 0        | —        |
| Trichophyton sp            | 3     | 0—5 100  | 21.2     | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| Tr. viride                 | 5     | 0—6 200  | 24.2     | 19       | 0—2 100 | 40.0     | 0        | —        | 0.0    | 0        | —        |
| Trichosporonoides sp       | 1     | 0—1 200  | 3.0      | 0        | —      | 0.0      | 0        | —        | 0.0    | 0        | —        |
| myc.ster. and unidentified | 34    | 0—10 000 | 54.5     | 1 100    | 95—26 000 | 100.0    | 0        | —        | 0.0    | 0        | —        |
| Yeasts                     | 55    | 0—12 000 | 60.6     | 13       | 0—760  | 40.0     | 36       | 0—1 300  | 50.0   | 36       | 0—1 300  | 50.0    |

A. = Aspergillus, Aur. = Aureobasidium, B. = Botrytis, M. = Micropolyspora, P. = Paecilomyces, S. = Scopulariopsis, T. = Thermoactinomyces, Th. = Thermomonospora, Tr. = Trichoderma, myc.ster. = mycelia sterilia
farmer’s lung, as described by Gregory & Lacey (1963).

The hay samples in this study included both good quality and extensively moldy batches, which indicates great variation in the microbiological quality of baled hay on different farms. The presence of Cladosporium and Alternaria species, however, indicates that the storage of hay probably promotes less microbiological deterioration than the storage of straw. If weather conditions during hay-making are unfavourable, the high moisture content of the hay allows the development of abundant thermotolerant and thermophilic microflora, e.g. A. fumigatus, T. vulgaris and M. faeni. The last-mentioned species requires a fairly high moisture content (47 %) of the material to grow (Cross et al. 1968). Such a high moisture content is rare in the climatic conditions of Finland, which may explain the rare occurrence of M. faeni in Finnish hay samples (Kotimaa et al. 1983, Mustonen et al. 1984). However, if hay is baled, the moisture content of hay is more critical as to moulding, because less water evaporates from tightly baled hay than from loosely collected hay.

Evidently, the grain material of this study was of good microbiological quality, although the samples were dried by forced unheated air. The level of exposure has been found to be higher during the handling of cool-air-dried grain compared to the handling of grain preserved and stored with other methods, e.g. drying with heated forced air (Mustonen et al. 1983). Our results imply that, at least in dry threshing season, cool air drying may be effective enough to prevent moulding of grain. There were only few thermophilic Streptomyces species, which are characteristic of self-heated grain (Festenstein et al. 1965). Species that occurred frequently, and in great amounts, in all the investigated materials were the fungi of the genera Aspergillus, Penicillium and Mucor, and thermophilic actinomycetes from the genus Streptomyces (especially in hay and straw) and T. vulgaris.

The diagnosis of farmer’s lung disease is based on symptoms, radiographic findings, lung function tests and the presence of microbial antibodies in serum (Rylander 1985). An antigen panel of four microbes (A. umbrosus, A. fumigatus, T. vulgaris and M. faeni) is used in serological tests for suspected cases of allergic alveolitis in Finland (Terho 1978, Husman et al. 1987). The present results show that different species of Penicillium, Aspergillus, Mucor, and Streptomyces are at least as important as the above-mentioned species in the exposure occurring in agricultural working environments. It has been assumed that thermophilic actinomycetes would be more potent in causing allergic alveolitis than other microbes involved in moulding (Wardrop et al. 1977). There have been cases of allergic alveolitis where the aetiological agents have been mesophilic fungi (Terho & Lacey 1979), e.g. spores of Penicillium (Fergusson et al. 1984, Solley & Hyatt 1980). Thus any kind of moulding causing high concentrations of airborne spores should be considered an undesirable phenomenon.

References

Andersen, A.A. 1958. New sampler for the collection, sizing and enumeration of viable airborne particles. J. Bacteriol. 76: 471—484.

Asmundsson, T., Gunnarsson, E. & Johannesson, T. 1983. ‘Haysickness’ in Icelandic horses: Precipitin tests and other studies. Equine Vet. J. 15: 229—232.

Cross, T., Maciver, A.M. & Lacey, J. 1968. The thermophilic actinomycetes in mouldy hay: Micropolyspora faeni sp. nov. J. Gen. Microbiol. 50: 351—359.

Corbaz, R., Gregory, P.H. & Lacey, M.E. 1963. Thermophilic and mesophilic actinomycetes in mouldy hay. J. Gen. Microbiol. 32: 449—455.
SELOSTUS

Varastoidun heinän, viljan ja oljen käsittelystä aiheutuva itiöaltistus

Marjut Kotimaa

_Kuopion alueyöterveyslaitos,_  
_PL 93, 70701 Kuopio_

Työssä tutkittiin tavanomaisissa tilaadolosuhteissa heinän, viljan ja kuivikkeina käytettävien olkien käsittelyn aiheuttamaa homepolyyaltistusta sisäruokintakauden lopulla. Ilmanäytteet mikroben määrittämiseksi kerättiin kuusivaihe-impaktoria (malli 10-800, Andersen Inc.) käytäen. Kuivikkeolkien (n = 5) aiheuttama homepolyyaltistus (3.7x10⁶ cfu/m³) oli merkittävästi suurempi kuin heinien (n = 33) (0.6 x 10⁶ cfu/m³) tai rehuviljan (u = 2) (0.2x10⁶ cfu/m³). Spontaania lämpennemistä osoittavien termotoleranttien sienten ja termofiilisten aktinomyyketien esiintyminen oli ominaista oljille. Heinästä irronneiden termotoleranttien sienten määrä oli vain noin 10 % ja viljasta irronneiden alle 1 % olkeen verrattuna, termofiilisten aktinomyyketten itiöitä irtosi heinästä vastaavasti noin 5 % ja viljasta 0.4 % oljesta irronneisiin määrin verrattuna. 

Kuivikkeolkien aiheuttaman itiöaltistuksen valtalaji oli _Thermoactinomyces vulgaris_, heinän ja viljan puolestaan _Aspergillus umbrosus_. Kaikissa materiaaleissa esiintyi runsaasti erilaisia varastosieninä tunnettuja _Aspergillus_ - ja _Penicillium_ -suvun lajeja (mm. _Aspergillus (A.) fumigatus_, _A. ochraceus_, _A. flavus_, _A. repens_, _A. versicolor_, _Penicillium (P.) expansum_, _P. piceum_, _P. citrinum_, _P. brevicipactum_, _P. echinulatum_, _P. verrucosum var. cyclopium_). Heinissä esiintyi tyypillisesti myös ns. peltosieninä pidettyjä _Cladosporium_-suvun lajeja, kuten _Cladosporium (C.) herbarum_, _C. cladosporioides_ ja _C. macrocarpum_. Saadut tulokset osoittavat, että rehujen ja kuivikkeiden käsittely maataloudessa altistaa perinteisesti homopölykeuhkon aiheuttajina tunnettujen mikroben lisäksi mm. monille _Penicillium-suvun_ homelle. Kuivikkeolkien mikrobiologinen laatu oli huono heinään ja viljaan verrattuna, heinän laatuvaihtelut oli suurta. Tulokset tukevat sitä käsitystä, että varastokuivureita tarvitaan sekä heinän että kuivikkeolkien kuivaamiseen, jotta näiden materiaalien homehtumisriski ja altistumisen aiheuttamat terveysriskit voitaisiin minimoida.