

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 (3.7×10^6 cfu/m³ air) in comparison to hay samples ($n = 33$) and grain samples ($n = 2$), which liberated 0.6×10^6 cfu/m³ and 0.2×10^6 cfu/m³, 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 %. *Thermoactinomyces 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, WISEMAN 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. Thirtythree 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 lightmicroscope 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 m³ during the handling of various materials on farms.

Microbe group	Material		
	Hay × 10 ³	Straw × 10 ³	Grain × 10 ³
Mesophilic fungi range	380 (9.7—6600)	1900 (520—6500)	160 (38—650)
Thermotolerant fungi range	24 (0.05—2000)	230 (12—2600)	1.6 (0.25—11)
Thermophilic actinomycetes range	36 (0.07—5100)	670 (100—3300)	1.6 (0.39—11)
Total range	630 (19—14000)	3700 (2100—12000)	160 (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 umbrinosus* 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 ($F=3.93$, $p<0.05$, $X^2=4.75$, $p<0.10$) (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 ($F=4.35$, $p<0.05$, $X^2=6.12$, $p<0.05$).

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

A. = *Aspergillus*, *Aur.* = *Aureobasidium*, *B.* = *Botrytis*, *M.* = *Micropolyspora*, *P.* = *Paecilomyces*, *S.* = *Scopulariopsis*, *T.* = *Thermomomomycetes*, *Th.* = *Thermomomomycetes*, *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. umbrinosus*, *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* (FERGUSON et al. 1984, SOLLEY & HYATT 1980). Thus any kind of moulding causing high concentrations of airborne spores should be considered an undesirable phenomenon.

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SELOSTUS

Varastoidun heinän, viljan ja oljen käsittelystä aiheutuva itiöaltistus

Marjut Kotimaa

Kuopion aluettyöterveyslaitos,

PL 93, 70701 Kuopio

Työssä tutkittiin tavanomaisissa tilaolosuhteissa heinän, viljan ja kuivikkeina käytettävien olkien käsittelyn aiheuttamaa homepölyaltistusta sisäruokintakauden lopulla. Ilmanäytteet mikrobien määrittämiseksi kerättiin kuusivaihe-impaktoria (malli 10-800, Andersen Inc.) käyttäen. Kuivikeolkien ($n = 5$) aiheuttama homepölyaltistus (3.7×10^6 cfu/m³) oli merkittävästi suurempi kuin heinien ($n = 33$) (0.6×10^6 cfu/m³) tai rehuviljan ($n = 2$) (0.2×10^6 cfu/m³). Spontaania lämpenemistä osoittavien termotoleranttien sienten ja termofiilisten aktinomykeettien esiintyminen oli ominaista oljille. Heinästä irronneiden termotoleranttien sienten itiöiden määrä oli vain noin 10 % ja viljasta irronneiden alle 1 % olkeen verrattuna, termofiilisten aktinomykeettien itiöitä irtosi heinästä vastaavasti noin 5 % ja viljasta 0.4 % oljesta irronneisiin määriin verrattuna.

Kuivikeolkien aiheuttaman itiöaltistuksen valtalaji oli *Thermoactinomyces vulgaris*, heinän ja viljan puolestaan *Aspergillus umbrosus*. Kaikissa materiaaleissa esiintyi run-

saasti 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. brevicompactum*, *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 homepölykeuhkon aiheuttajina tunnettujen mikrobien lisäksi mm. monille *Penicillium*-suvun homeille. Kuivikeolkien mikrobiologinen laatu oli huono heinäan ja viljaan verrattuna, heinäan laatu vaihtelu oli suurta. Tulokset tukevat sitä käsitystä, että varastokuivureita tarvitaan sekä heinäan että kuivikeolkien kuivaamiseen, jotta näiden materiaalien homehtumisriski ja altistumisen aiheuttamat terveysriskit voitaisiin minimoida.