Farmer's lung in a group of Scottish dairy farms

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ABSTRACT The microbiology of the air of byres and bruising sheds and of hay, grain and dust from bruising machines was studied in 12 dairy farms in Ayrshire and one in Perthshire. Seven farms (FLD) had a known case of farmer's lung disease and five farms (non-FLD) were free from the disease. Concentrations of mesophilic organisms and of thermotolerant and thermophilic fungi did not vary significantly between the two types of farm but the concentrations of thermophilic actinomycetes and bacteria, notably *Micropolyspora faeni*, were higher in general on FLD farms. Culture filtrate and mycelial extracts of the most commonly isolated organisms were tested against three groups of sera (11 from patients with farmer's lung disease, 14 from healthy personnel on FLD farms and 13 from personnel without farmer's lung disease on non-FLD farms). Only extracts from a *Penicillium* sp. and a *Streptomyces* sp. precipitated with a number of sera, when extracts from the 12 most commonly isolated fungi, from six thermophilic actinomycetes other than *M. faeni* and *Thermoactinomyces vulgaris*, and from two thermophilic bacteria were tested. There was no correlation between disease and seropositivity. All sera reacted to at least one of 60 carbol-saline and trichloroacetic acid extracts from 30 samples of hay, grain and dust. Although sera from personnel on both FLD and non-FLD farms precipitated with 16% and 19% of these extracts respectively, reactivity to extracts from FLD farms was greater on average than to those from non-FLD farms. When tested by several serological methods against extracts of a type culture of *M. faeni* and by double diffusion against farmer's lung hay (FLH) and extracts of local isolates of *M. faeni*, 91% of all clinical cases of farmer's lung were serologically positive but no one test was adequate for determining sensitisation. Fifty-four per cent of sera from FLD and also from non-FLD farms were positive in at least one test. Sixty-nine per cent of seropositive personnel on FLD farms were also symptomatic. The occurrence of symptoms apparently correlated with the higher concentrations of *M. faeni* encountered on FLD farms.

The pilot survey by Grant *et al.* (1972) on farmer's lung disease in Scotland, reported prevalences of 86 and 23/1000 of the farming populations surveyed. Ayrshire in the West of Scotland gave the higher of the two prevalences and was therefore chosen for intensive study. Farm products from this area also yielded higher numbers of thermophilic actinomycetes than equivalent samples from East Lothian which had the lower incidence of disease (Wardrop, 1974). The evidence that the antigens provoking disease occur in thermophilic actinomycetes, notably *Micropolyspora faeni* and to a lesser extent *Thermoactinomyces vulgaris*, is regarded as convincing (Pepys *et al.*, 1962; Pepys and Jenkins, 1965;

Barbee *et al.*, 1965). Sera from a variable number of patients are also known to contain precipitins to a range of fungi from genera such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor* and *Humicola*. The significance, if any, of these precipitins in relation to disease is obscure. The sources of respirable organic dusts containing antigens which induce extrinsic allergic alveolitis in the farm environment are products such as hay, grain and dust generated by bruising and milling machinery. The present investigations were undertaken to compare the microbiological components of farms with (FLD) and without (non-FLD) clinical cases of farmer's lung disease.

As little is known about the significance of the microbiology of Scottish farms in relation to respiratory disease, we hoped to relate the occurrence

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of commonly encountered mesophilic, thermo-tolerant and thermophilic organisms to the incidence of serum precipitins in diseased and healthy personnel. Additional studies involved the reactivity of sera to extracts from farm products.

The survey was carried out during March and April 1972 following a very good hay-making season and average grain harvest in 1971. The hay which was fed to animals had therefore been stored for eight to nine months and the grain for seven to eight months. Individuals working with these materials had been in contact with them and exposed to their antigenic content, if any, for a period of five to six months during the winter while feeding stock.

Materials and methods

SAMPLING OF FARM ENVIRONMENTS AND PRODUCTS

In Ayrshire, six dairy farms each with a known case of farmer's lung disease (FLD farms) and five dairy farms with no record of disease (non-FLD farms) in the six years previous to 1972, were chosen for microbiological examination during March and April 1972. One Perthshire dairy farm with a known case of farmer's lung was also studied. The 12 farms were of equivalent size (100-210 acres) and similar in agricultural practice.

The air in dairy byres of five FLD farms (LM, SB, MH, KM and A) and five non-FLD farms (MF, MT, WH, BM and B) in Ayrshire was sampled before and approximately five minutes after hay unbaling and dairy cattle foddering. The air in bruising sheds of four FLD farms (SB, EM, KM and A) and four non-FLD farms (MF, WH, BM and B) was sampled before and approximately five minutes after barley bruising or hammer-milling had begun. Samples were taken from the dairy byre and bruising shed of the farm (BP) in Perthshire before and after hay unbaling and before and after oats bruising respectively.

For sampling, an eight-stage Andersen Sampler (Andersen, 1958) was run at an air flow of 25 l/min for periods of between five and 30 seconds. The apparatus was loaded with eight disposable 9 cm Petri dishes and sampled air in farm buildings before (time A) and after (time B) hay unbaling or grain bruising. At time A the upper four plates (0-3) contained 2% malt agar (M) and the lower four, which had three compartments, contained half-strength nutrient broth (Oxoid) with 500 mg/l cycloheximide (N + C), glycerol asparaginate (GA) and tap water (TW) in 2% agar media. Two sets of eight plates were successively exposed at time B. One was a set of malt agar plates subsequently incubated at 26°C,

and the other was a set of three-compartment plates containing media as described above and incubated at 46°C.

Samples of hay, whole grain, bruised or hammer-milled grain and dust off bruising and milling machines were collected at the time of unbaling, bruising or milling. They were placed in weighed sterile four-litre tin cans and the weight of each sample was determined. By means of a length of stiff polythene tubing connected to a modified tin lid, each sample was attached in turn to the sampler and shaken vigorously on a Griffin flask shaker for 30 seconds. Air was pumped out of the tin over the plates in the sampler until all air containing propagules released from the sample was removed and the tin buckled. Of two sets of plates used to sample each product, one containing 2% malt agar was incubated at 26°C; the other, consisting of three-compartment plates with media as described above, was incubated at 46°C.

COUNTING AND IDENTIFICATION OF COLONIES OF FUNGI AND ACTINOMYCETES

Total counts of colonies on all plates were made after 48 h according to the positive hole conversion and microscope method of Andersen (1958). Mesophilic fungi on malt plates were identified after seven to 10 days. Thermophilic fungi on GA plates were identified after four to five days, or were sub-cultured and grown for a further 10 to 14 days. The more common thermophilic actinomycetes were identified to generic level: *Thermoactinomyces vulgaris* and *Micropolyspora faeni* were identified using the criteria of Cross (1968) and Cross *et al.* (1968). The numbers of different types of culturable propagules isolated per cubic metre of air or per 10 g of hay, grain or dust were calculated.

EXTRACTS FROM MICROORGANISMS

Culture filtrate (CF) and mycelial (M) extracts were obtained from shake cultures of the fungi and actinomycetes most commonly found on both FLD and non-FLD farms. Fungi were grown in 400 ml aliquots of Czapek-Dox (Oxoid) containing 2 g Nutrient Broth (Oxoid) in litre flasks shaken in darkness in an orbital incubator. Mesophilic fungi were grown for six weeks at 26°C, thermophilic and thermotolerant fungi for three weeks at 46°C. Thermophilic actinomycetes were grown under similar conditions for three weeks at 46°C in nutrient broth containing 1% glucose. Culture filtrate and mycelial fractions were obtained as shown in Figure 1.

EXTRACTS FROM HAY, GRAIN AND DUST

Samples of 5 g hay or dust and of 10 g whole or

Fig. 1 Flow chart of antigen extraction from fungi and actinomycetes.

processed grain were placed in litre screw-topped jars. The samples were defatted in 200 ml acetone overnight and after decanting, the acetone was passed through muslin to retain any large particles. Two hundred ml of Coca's solution were added to each of the samples and the jars were shaken in an orbital incubator for seven days at 26°C. Filtered solutions were dialysed for 72 h against running tap water, concentrated against polyethylene glycol 4000 and dried by rotary evaporation. The product was reconstituted, by addition of distilled water, to a concentration of 20-25 mg/ml for use in double diffusion tests and 50-60 mg/ml for use in immunoelectrophoresis.

Samples of 5 g hay or dust and of 10 g whole or processed grain were placed in litre screw-topped jars. After 200 ml 5% trichloroacetic acid (TCA) had been added to each sample, they were shaken for three days in an orbital incubator at 26°C. Further processing was as described above for extraction using Coca's solution.

FARMER'S LUNG HAY ANTIGEN (FLH)

This was supplied by the Public Health Laboratory Service.

SEROLOGY

Blood samples (20 ml) were obtained from farmers, farm workers and farmers' wives actively involved

Fig. 2 The number of colonies of mesophilic organisms $\times 10^{-6}$ isolated per m^3 of air from dairy byres after unbaling hay (1), and from bruising sheds after bruising grain (2).

in work on nine FLD and five non-FLD farms. Two positive sera, one (B) from a patient in Perthshire and one (C) from a patient in Ayrshire were obtained from hospital clinics. Sera from a total of 11 FLD cases, 14 non-FLD personnel on FLD farms and 13 non-FLD personnel on non-FLD farms were tested. A control group of 50 sera was obtained from the Blood Transfusion Service, Edinburgh.

Double diffusion (DD) in agar was carried out as described by Ouchterlony (1953). Immunoelectrophoresis (IE) was by the method of Pepys and Jenkins (1965) and the numbers of arcs of precipitation were classified accordingly. Immunoelectrophoresis (IEO) was conducted according to the method of Gordon *et al.* (1971).

Results

ORGANISMS IN THE AIR OF FARM BUILDINGS

After hay unbaling and grain bruising, more mesophilic organisms were found per m^3 of air than before. With four exceptions, the concentrations of thermophilic actinomycetes and bacteria were also higher after unbaling and bruising than before. There was considerable variation in the numbers of colonies of mesophiles (Fig. 2) isolated from dairy byres on FLD farms, but the numbers from all non-FLD dairy byres were of approximately the same magnitude, intermediate to the values obtained from the air of FLD dairy byres. Although the

Fig. 3 The number of colonies of thermophilic actinomycetes and bacteria (a) and of *M. faeni* (b) $\times 10^{-3}$ isolated per m^3 of air from dairy byres after unbaling hay (1), and from bruising sheds after bruising grain (2).

highest level in dairy byres was $5418 \times 10^3/m^3$, values of between 8000×10^3 and $88\,861 \times 10^3/m^3$ were recorded in five bruising sheds.

The corresponding results for thermophilic actinomycetes and bacteria are shown in Fig. 3(a). It is notable that in four of the 11 dairy byres sampled, high values of between 402×10^3 and $796 \times 10^3/m^3$ were recorded, but only one high value was from a non-FLD farm (MT). Two FLD farms, however, yielded very high counts of 1663×10^3 and $1811 \times 10^3/m^3$ from the air of their bruising sheds, while all counts from non-FLD farms were relatively low. The results for *M. faeni* are shown in Fig. 3(b). Values above 45×10^3 propagules/ m^3 were recorded only on FLD farms with a maximum of $427 \times 10^3/m^3$ for the bruising shed of farm KM. These results suggest that significantly higher levels of *M. faeni* are found on FLD farms. *T. vulgaris* was less common than *M. faeni*, the highest value being $57 \times 10^3/m^3$ from the dairy byre of farm A and in only three of the bruising-sheds of nine farms was any value recorded, the maximum being $7 \times 10^3/m^3$. Thermophilic fungi showed a highest count of $457 \times 10^3/m^3$ in the dairy byre of farm A. Only two bruising sheds, compared with four dairy byres, had values of between 30×10^3 and $79 \times 10^3/m^3$.

SPECIES AND GROUPS OF ORGANISMS ISOLATED

The mesophilic groups and species of fungi isolated from the air of farm buildings and from samples of hay, grain and dust were as follows: *Absidia* spp., *Acremonia atra*, *Alternaria* spp., *Aspergillus candidus*, *A. flavus*, *A. fumigatus**, *A. glaucus**, *A. nidulans**, *A. niger*, *A. ochraceus*, *A. ornatus*, *A. restrictus**, *A. terreus**, *A. ustus*, *A. versicolor**, *Aureobasidium pullulans*, *Botryotrichum* spp., *Botrytis cinerea*, *Chaetomium* spp., *Chrysosporium* spp., *Cladosporium* spp.*, *Doratomyces* sp., *Epicoccum nigrum*, *Fusarium* spp., *Geotrichum* spp., *Mucor* spp., *Mycelia sterilia*, *Paecilomyces* spp., *Penicillium* spp.*, *Rhizopus* spp.*, *Scopulariopsis* spp., *Sporothrix* sp., *Trichoderma viride*, *Trichothecium roseum*, *Verticillium* spp. and Yeasts. Species marked * were used for serological testing. The Aspergilli were divided into the collective Groups described by Raper and Fennell (1965).

Thermophilic and thermotolerant fungal species and groups comprised *A. fumigatus*, *Humicola* spp., and Mucorales. Those used for serology were two strains of the *A. fumigatus* Group, a thermophilic *Humicola* sp. and *Mucor pusillus*. Commonly isolated thermophilic actinomycetes included *Saccharomonospora viridis* (Nonomura and Ohara, 1971), *Streptomyces* spp., *Thermoactinomyces vulgaris* and *Micropolyspora faeni*. Organisms extracted for serology were six isolates of *M. faeni* from different farms, four isolates of a variant of *M. faeni*, five *Streptomyces* spp., one isolate of *S. viridis*, one isolate of *T. vulgaris*, an isolate of *Bacillus cereus* var. *mycoides* and one of a Gram-negative thermophilic bacterium. Culture filtrate and mycelial extracts were also prepared from Type cultures of *M. faeni* and *T. vulgaris*.

SEROLOGICAL REACTIONS TO MICRO-ORGANISMS OTHER THAN *M. FAENI* AND *T. VULGARIS*

Table 1 shows all positive reactions between 36 FLD and non-FLD sera and CF and M extracts from a range of fungi and two thermophilic *Streptomyces* spp. Extracts from only two organisms reacted widely within all groups of sera; the M extract of the *Penicillium* sp. and the CF extract of a white, thermophilic *Streptomyces* sp. reacted to a total of 10 and 14 sera respectively. There was no correlation between FLD and reactions to any of the organisms tested.

SEROLOGICAL REACTIONS TO EXTRACTS OF HAY, GRAIN AND DUST

Results are summarised in Tables 2 and 3. Of the sera tested to both Coca and TCA extracts of hay

Table 1 Positive reactions of 36 sera to culture filtrate (CF) and mycelial (M) extracts of microorganisms

Serum		Mesophilic fungi								Thermophilic fungi					Actinomycetes	
Group	No.	Rhiz.	Clad.	Pen.	Asp.f. (i)	Asp.t.	Asp.n.	Asp.r.	Asp.v.	Asp.f. (ii)	Asp.f. (ii)	Asp.f. (iii)	Hum.	Mucor	103	309
		CF	CF	M	CF	M	M	M	CF	CF	M	M	M	M	CF	CF
FLD	2					+		+				+		+	+	
	21														+	
	28			+												
	B			+			+					+	+			
	C			+											+	
Non-FLD	1														+	
on FLD	8			+												
farms	9	+	+													
	11														+	
	12														+	
	27			+											+	
	29			+											+	
	30														+	
	32														+	
	33				+										+	
Non-FLD on	3										+			+	+	+
non-	4														+	
FLD	5			+											+	
farms	6			+				+							+	
	14														+	
	17			+												
	25			+												

Rhiz. = *Rhizopus* sp.; Clad. = *Cladosporium* sp.; Pen. = *Penicillium* sp.; Asp.f. = *Aspergillus fumigatus*; Asp.t. = *Aspergillus terreus*; Asp.n. = *Aspergillus nidulans*; Asp.r. = *Aspergillus restrictus*; Asp.v. = *Aspergillus versicolor*; Hum. = *Humicola* sp.; Mucor = *Mucor* sp.; 103 = White thermophilic actinomycete; 309 = Brown thermophilic actinomycete; (i) = Mesophilic grown at 26°C; (ii) = Thermotolerant grown at 46°C (strain 1); (iii) = Thermotolerant grown at 46°C (strain 2); FLD = Farmer's lung disease.

Table 2 The microbiology of hay samples in relation to serology

Hay sample	Total no. colonies (M; 26°C)	Dominant groups or spp. (M; 26°C)	Total no. colonies per 10 g (N + C; 46°C)	Total no. colonies M. faeni per 10 g (N + C; 46°C)	Percentage of 36 sera precipitating with material extracted by Coca TCA	
LM* (G)	71	<i>A. glaucus</i> <i>Trichoderma</i> sp.	2959	2862	75	47
KM* (B)	3634	Yeasts Actinomycetes	2550	2450	22	33
BP* (B)	17947	Yeasts <i>A. fumigatus</i> Mucorales Actinomycetes	12298	6143	28	53
MH* (B)	4036	<i>A. versicolor</i> <i>A. nidulans</i> <i>Penicillium</i> spp.	1107	14	19	11
Average					36	36
MF (G)	229	<i>A. glaucus</i>	6	0	14	17
WH (G)	45	<i>A. glaucus</i>	10	6	6	39
B (G)	1602	<i>A. restrictus</i> Actinomycetes	10	1	6	3
MT (G)	911	<i>A. restrictus</i> <i>A. versicolor</i>	95	69	14	3
BM (G)	3715	<i>A. versicolor</i>	200	38	0	3
Average					8	13

M = malt agar; N + C = nutrient agar containing cycloheximide; Coca = Coca's solution; TCA = trichloroacetic acid; * = sample from FLD farm; (B) = visually bad; (G) = visually good.

from FLD farms, on average 36% were positive whereas only 8% and 13% respectively were positive to similar extracts from hay collected on non-FLD farms. Reactivity largely correlated with high values

for total colonies of thermophilic organisms and of *M. faeni* but was not related to total numbers of colonies of mesophiles. Similar but less marked differences between extracts from FLD and non-

Table 3 The microbiology of whole barley, processed barley and dust samples in relation to serology

Sample	Total no. colonies (M; 26°C)	Dominant groups or species (M; 26°C)	Total no. colonies/10 g (N + C; 46°C)	Total no. colonies M. faeni (N + C; 46°C)	Percentage of 36 sera precipitating with material extracted by Coca TCA	
Whole barley						
SB*	5359	<i>Penicillium</i> spp.	802	618	31	17
KM*	2556	<i>A. candidus</i>	2334	1821	47	6
		Actinomycetes				
EM*	7076	<i>Penicillium</i> spp.	271	1	11	22
		Yeasts				
A*	28	Yeasts	5	1	3	3
BP* (oats)	509	<i>Penicillium</i> spp.	179	13	3	0
		Yeasts				
Average					19	10
WH	5	Yeasts	125	60	14	6
B	2273	<i>Penicillium</i> spp.	160	46	3	6
		<i>Geotrichum</i> sp.				
		Yeasts				
MF	5796	<i>Penicillium</i> spp.	8	0	8	3
		Yeasts				
Average					8	5
Processed barley						
SB*	11781	<i>Penicillium</i> spp.	1576	1182	3	0
A*	2250	Bacteria	132	40	3	3
EM*	21	Yeasts	3	0	33	0
		<i>Geotrichum</i> sp.				
Average					13	1
MF	3384	<i>Penicillium</i> spp.	1	0	3	0
		<i>Geotrichum</i> sp.				
		<i>Paecilomyces</i> spp.				
B	4355	<i>Geotrichum</i> sp.	21	0	3	0
		Bacteria				
Average					3	0
Dust						
SB*	1689	Yeasts	430	73	42	6
EM*	2	<i>Penicillium</i> spp.	239	0	28	33
KM*	54	Actinomycetes	46	28	28	14
A*	28	<i>Penicillium</i> spp.	20	1	25	0
BP*	2	<i>Penicillium</i> spp.	6	0	6	6
Average					26	12
MF	16	<i>Geotrichum</i> sp.	26	0	6	6
WH	874	<i>Penicillium</i> spp.	2	1	39	17
		Yeasts				
B	6713	Yeasts	27	4	47	11
		<i>Penicillium</i> spp.				
		<i>Geotrichum</i> sp.				
Average					31	11

M = malt agar; N + C = nutrient agar containing cycloheximide; Coca = Coca's solution; TCA = trichloroacetic acid; * = sample from FLD farm.

FLD farms were obtained for whole barley samples. The effect of processing was demonstrated by the very low reactivity of extracts from processed barleys. Extracts from dusts, however, reacted more frequently with sera than those from whole barley on both FLD and non-FLD farms.

Table 4 shows that all sera were precipitin-positive to at least one of 60 extracts (Coca and

TCA) of 30 samples of hay, grain and dust. A larger percentage of sera from non-FLD farms was positive to a higher number of extracts than in the other 2 groups. Non-FLD sera from FLD farms reacted to the lowest number of extracts. It was calculated that on average sera from FLD farms reacted to 16% of all extracts whereas sera from non-FLD farms reacted to 19%. Twenty-three

Table 4 Percentage sera positive to extracts of hay, grain and dust in relation to positivity to *M. faeni*

Number of extracts from hay, grain and dust with which sera reacted	Percentage sera positive to hay, grain and dust			Percentage sera positive to <i>M. faeni</i>		
	FLD (10)	non-FLD on FLD farms (14)	non-FLD on non-FLD farms (12)	FLD (11)	non-FLD on FLD farms (14)	non-FLD on non-FLD farms (13)
1-10 out of 60	80	93	59	64	28	7.5
11-20 " " "	0	7	33	0	0	31
36 " " "	10	0	0	9	0	0
30 " " 38	0	0	8	0	0	7.5
29 " " 36	10	0	0	9	0	0
Totals	100	100	100			
Not tested to hay, grain and dust, but to <i>M. faeni</i>				9		7.5
Totals				91	28	54

Table 5 The reactions of 38 sera to farmer's lung hay (FLH) antigen and to antigens from a type culture and local Ayrshire isolates of *M. faeni*

Serum reaction category	No. and sources of sera			IE	Double diffusion			IFAT	IEO
	FLD	Non-FLD on FLD farms	Non-FLD on non-FLD farms		FLH	Type <i>M. faeni</i>	Local <i>M. faeni</i>		
1	3			A, B, C A, B, C A, B, C A, B A, B, C A, B, C	+(3) +(4) +(6) +(3) +(6) +(5)	CF +(2) +(4) +(4) +(1) +(4) +(2)	CF +5/6 NT +6/6 +5/6 NT +6/6	M NT NT +6/6 +5/6 NT +6/6	++ ++ NT ++ ++ ++ ++
2	3				+(1) +(1) +(1) +(2) +(1) +(2)		+1/6 +6/6 +1/6 +1/6 +2/6 +2/6	+1/6 +5/6 +2/6 +1/6 +2/6 +2/6	+ + + + + ++
3	4			A			+6/6 +5/6		
		2			+(1)				
			1				NT NT		++ ++
4	1			A	+(1)	+(1)		+	+
Negative	1	10	6						

IE = immunoelectrophoresis; A, B and C = arcs of precipitation according to Pepys and Jenkins (1965); IEO = immunoelectroosmophoresis; IFAT = indirect fluorescent antibody test; CF = culture filtrate; M = mycelial extract; (1) = one precipitin arc formed; 1/6 = one positive reaction to six extracts; NT = not tested; FLD = farmer's lung disease.

per cent of FLD sera reacted, as did 11% of non-FLD sera from personnel on FLD farms. Two FLD sera and one non-FLD serum reacted to 81%, 60% and 79% respectively of extracts.

SEROLOGICAL REACTIONS TO FLH, *M. FAENI* AND *T. VULGARIS*

All reactions of sera to FLH and to antigens from the Type culture and strains of *M. faeni* are shown in Table 5. Four categories of positive sera were distinguished. Of the six strongly reactive sera comprising Category 1, all but one produced A, B

and C arcs of precipitation in immunoelectrophoresis and also precipitated with extracts of the Type strain of *M. faeni*. A second category of seven sera, negative in IE and in tests with the *M. faeni* Type strain were positive to extracts from local strains of the organism. The seven sera comprising Category 3 were very weakly reactive, each giving a positive result in one test only. The single serum in Category 4 was weakly reactive in all tests except those involving local *M. faeni* isolates. There was a marked lack of reactivity in the sera from non-FLD personnel on FLD farms, 10 being negative for all tests

Table 6 The serology of farmer's lung patients and controls in relation to the occurrence of *M. faeni* in farm environments, hay and grain

FLD farms									Non-FLD farms					Totals % Sero-positive	
LM	SB	MH	KM	A	EM	BP	Oth.	MT	MF	WH	BM	B			
FLD patients (T)	1	1	1	1	1	1	1	3						10	90 } 54
on FLD farms (S)	0	1	1	1	1	1	1	3						9	
Non-FLD on (T)	7	1	0	1	2	0	0	3						14	
FLD farms (S)	1	0	0	1	0	0	0	2						4	
Non-FLD on (T)									2	4	2	3	2	13	29 } 54
non-FLD farms (S)									2	1	1	2	1	7	

Density of *M. faeni*

Air after unbaling hay	++++	-	++	-	++	NT	-	+	-	-	-	-
On hay	++++	-	-	+++	-	NT	++++	+	-	-	+	-
Air after grain bruising	NT	+++	NT	++++	-	-	-	NT	++	+	-	-
On grain	NT	+++	NT	++++	-	++	-	NT	-	+	-	+

(T) = total number; (S) = number serologically positive to antigens of *M. faeni* and FLH; NT = not tested; Oth. = other sera.

Number of colonies of *M. faeni*:

Air sampling	Hay and grain sampling
- = up to 6/l	up to 15/10 g
+ = 10-15/l	30-70/10 g
++ = 30-60/l	160/10 g
+++ = 90/l	600-1300/10 g
++++ = 300-430/l	1800-3200/10 g

(Values for hay from MH, A, KM and BP are averages of two samples.)

out of 14. Although no sera reacted to the M extract from the *T. vulgaris* Type culture, six reacted to the equivalent CF of this organism. Five of these six also produced between two and six arcs of precipitation with FLH. Neither M nor CF extracts of an isolate of *T. vulgaris* from Ayrshire precipitated with sera.

ENVIRONMENTAL HAZARD IN RELATION TO SEROLOGY

All FLD patients worked with both hay and grain. Hay alone was considered to be the main hazard by four patients, hay and grain together, by five and grain alone by the remaining two. Four blamed shovelling or bagging of grain in bulk at harvest time for their symptoms and five blamed bruising or milling stored grain. There was no correlation between the alleged hazards and serological reactivity of individuals to extracts of hay, grain and dust, or to those of *M. faeni* or *T. vulgaris*.

Of 14 non-FLD personnel on FLD farms, three were rarely in contact with hay or grain, 10 were regularly in contact with hay, five were regularly involved in bruising or milling grain and six were regularly in contact with grain only when feeding cattle. Of the 13 personnel on non-FLD farms, 12 were regularly in contact with hay, and four were regularly involved in bruising or milling grain. Three more occasionally bruised or milled grain while four were in contact with grain only when feeding animals. There was no correlation between the

degree of serological reactivity of non-FLD personnel and their contact with hay or grain.

SEROLOGICAL REACTIVITY AND DISEASE: FARM PRODUCTS AND CONCENTRATION OF *M. FAENI*

The numbers of thermophilic actinomycetes and bacteria isolated per m³ of air after unbaling and bruising were higher from FLD farms than from non-FLD farms (Fig. 3(a)). Table 6 shows that high concentrations of *M. faeni* were isolated from air or from hay or grain samples in four of seven FLD farms but no comparable results were obtained from non-FLD farms. Serological positivity recorded in Table 6 indicates reactivity to FLH and/or any test for *M. faeni* and the results include those for sera associated with the environments sampled as well as for an additional three FLD and three non-FLD sera from FLD farms. Ninety per cent of FLD subjects and 29% of non-FLD personnel on FLD farms were serologically positive, giving a figure of 54% positive for all personnel. Fifty four per cent of all individuals on non-FLD farms were also positive. The fact that 69% of all sensitised individuals on FLD farms were also symptomatic suggests that the large numbers of colonies of *M. faeni* isolated from FLD farms were instrumental in inducing disease. However, extracts of hay, grain and dust from such farms precipitated with a larger proportion of sera than did those from non-FLD farms; this indicates the presence of some additional

reactive factors in the extracts, because reactivity did not correlate with that of *M. faeni* (Table 4). It is possible that 'D line' antigen, possessing determinants in common with β -teichoic acids (Faux *et al.*, 1970) may have been involved.

A notable but unexplained result is the remarkably low reactivity of sera from non-FLD personnel on FLD farms to FLH and extracts of farm products and *M. faeni* despite the high environmental concentrations of potentially antigenic propagules.

Discussion

Large concentrations of microorganisms were recovered from farm buildings after hay unbaling and grain bruising and from samples of these farm products. Although the concentrations of propagules released per unit fresh weight of hay were greater than for grain, the weight of grain processed in one bruising operation is generally greater than the weight of hay dealt with in one unbaling operation.

In general farm practice grain may be bruised every second or third day compared with twice-daily unbaling of hay, and the overall intake of respirable dust produced by the two processes over a period of weeks is possibly comparable, assuming that the materials are in an equivalent state of preservation. As the numbers of organisms recovered from samples of dust from bruising machinery were lower than expected, it is probable that a large proportion of propagules were dead but could still have been allergenic.

Thermophilic actinomycetes and bacteria, particularly *M. faeni*, were more common on farms where there was a case of farmer's lung than on those free from the disease. As no correlation could be established between concentrations of mesophilic organisms or of thermophilic fungi and the presence or absence of disease, these organisms may be considered to be of little significance. The results of serological tests, using extracts of the most common of these organisms confirmed this as few extracts, apart from those of a mesophilic *Penicillium* species, reacted with any of the groups of sera. Of the thermophilic actinomycetes other than *M. faeni* and *T. vulgaris* only one reacted with sera. The occurrence of precipitins to fungi was considerably lower than that reported by Pepys *et al.* (1963) and the results have clearly implicated thermophilic actinomycetes, particularly *M. faeni*, as the primary source of sensitising and/or disease-invoking antigens in the Scottish farming environment as in other similar environments in Great Britain. Although it is always assumed that high concentrations of *M. faeni* are necessary to provoke symptoms in sensitised individuals, little information has been made avail-

able previously in terms of propagules per unit volume of air in the home environments of specific cases of farmer's lung.

Storage of farm products at too high an initial water content leads to self-heating and the subsequent growth of thermophilic organisms, resulting in the production of FLH antigen (Pepys *et al.*, 1963; Festenstein *et al.*, 1965). Most of the antigenic content of FLH has been shown to occur in extracts of *M. faeni* which also yield the A, B and C arcs of precipitation characteristic of FLH in immunoelectrophoresis (Pepys *et al.*, 1963). It has been claimed that sera giving all three arcs are from patients having a history of frequent and severe attacks; Hollingdale and Murray (1974) found that sera from most severe cases cause precipitation of polysaccharide antigens in the C region. In the results reported here however two out of five sera giving C arcs of precipitation were from asymptomatic farmers. Pepys *et al.* (1962) noted that 80% of patients reacted to FLH, and Pepys and Jenkins (1965) quoted figures of 87% positive to extracts of *M. faeni*. In Scottish farms, however, Grant *et al.* (1972) recorded that only 43% of cases were positive. Hapke *et al.* (1968) established that 53% of patients in the chronic stage of disease were positive to *M. faeni*, compared with 100% in the acute stage. The validity of a positive test to FLH and extracts of *M. faeni* as a primary criterion in diagnosis has therefore been challenged (Grant *et al.*, 1972; Morgan *et al.*, 1973; Smyth *et al.*, 1975). It is concluded from the Ayrshire results that a positive serological reaction indicates sensitisation with or without disease.

The numbers of healthy farmers with precipitins to antigens of *M. faeni* have been reported as none (Boyd, 1971), 15-21% (Pepys and Jenkins, 1965) and 23% (Morgan *et al.*, 1973). In the present study, 30% of healthy individuals reacted with FLH in double diffusion tests and only 55% of those with disease were also positive. After using extracts of *M. faeni* and the fluorescent antibody test however the figure for all patients became 91% and for all healthy individuals was 41%. The use of a range of antigenic preparations from an isolate of *M. faeni* has been shown to be essential by Dick *et al.* (1973). Increased incidence of detection of antibody to *M. faeni* has been claimed using fluorescent antibody (Parratt and Peel, 1972; Boyd and Parratt, 1974) and radioimmunoassay (Parratt *et al.*, 1975) techniques. The present work has established the value of using several isolates of *M. faeni* from local farm environments in preparing serological reagents.

In an area of known high incidence of farmer's lung in the West of Scotland, even after a very good hay-making season the degree of preservation of hay

from FLD farms, as indicated by microbial evidence of self-heating, was generally not as good as that of hay from non-FLD farms. There was also an unexpectedly high percentage sensitisation of personnel on non-FLD farms to FLH and various *M. faeni* extracts. These results emphasise the need for efficient hay-making and preservation of grain combined with the judicious use of efficient respirators (Gourley and Braidwood, 1971) for protection against antigen.

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