ORIGINAL ARTICLES AAEM
Ann Agric Environ Med 2006, 13, 267–273

IMPACT OF AGRICULTURAL PRACTICES ON MICROBIOLOGY OF HAY, SILAGE AND FLOUR ON FINNISH AND FRENCH FARMS

Gabriel Reboux[1], Marjut Reiman[2], Sandrine Roussel[1], Kirsti Taattola[2], Laurence Millon[1], Jean-Charles Dalphin[3], Renaud Piarroux[1]

[1]Department of Mycology, University Hospital, Besançon, France
[2]Finnish Institute of Occupational Health, Kuopio, Finland
[3]Department of Respiratory Disease, University Hospital, Besançon, France

Reboux G, Reiman M, Roussel S, Taattola K, Millon L, Dalphin JC, Piarroux R: Impact of agricultural practices on microbiology of hay, silage and flour on Finnish and French farms. *Ann Agric Environ Med* 2006, **13**, 267–273.

Abstract: Exposure to microorganisms in farm environments may cause respiratory disorders, e.g. asthma, organic dust toxic syndrome and allergic alveolitis. By reducing microbiological deterioration of organic materials, some agricultural practices have a protective effect. Microbiological analyses were carried out on hay, silage and flour samples (n=107) from farms in Finland and France (n=23) that use different methods of haymaking. High concentrations of *Absidia corymbifera* were found in approximately 35% of French hay samples and only 10% of Finnish hay samples. Concentrations of *Eurotium* spp. were found in 20% of hay samples from both regions. High concentrations of *Wallemia sebi* typified Finnish hay (38%) more than French hay (8%). *Rhodotorula* yeast was frequently and abundantly found in Finland, but never in France. The method used to make hay appeared to be the main factor affecting the microbiology of the hay. *A. corymbifera* and *Eurotium* spp. concentrations were smaller in low-density square bales than in others. In conclusion, our results emphasize the importance of good agricultural practice in the microbiological quality of fodder.

Address for correspondence: Gabriel Reboux, Laboratoire de Parasitologie-Mycologie, CHU J. Minjoz, Boulevard Fleming, F-25030 Besancon, France.
E-mail: gabriel.reboux@ufc-chu.univ-fcomte.fr

Key words: farm work, agricultural practices, exposure, hay, fungi, actinomycetes.

INTRODUCTION

The farming environment is an important source of exposure to microorganisms and can cause several respiratory disorders, such as organic dust toxin syndrome (ODTS), asthma, chronic bronchitis, or extrinsic allergic alveolitis (EAA). These health problems arise from exposure to particles found in the air in cowsheds.

Farmer's lung disease (FLD) is an EAA caused by inhalation of certain actinomycetes or moulds during the winter handling of mouldy organic materials. Rainfall during harvest is the main contributing factor in the development of microorganisms in hay. Several studies have linked the incidence of FLD to weather conditions [21, 22]. Agricultural practices, e.g. mechanical drying of hay or grain, have a protective effect on respiratory health of farmers by reducing microbiological contamination [3]. An epidemiological study in Ireland showed the incidence of FLD to have remained constant over 13 years. The authors attributed this stability to the lack of development in agricultural techniques [15].

In Finland, several sources of exposure to mould in an agricultural environment have been recognised: hay, grain and occasionally even ensilage used as feed; straw, sawdust, wood shavings and peat used as bedding materials [11]. Modern farms typically use automated

Received: 24 March 2006
Accepted: 7 September 2006

working practices where possible, less hay is used for feeding, smaller amounts of bedding materials are being used, and mechanically ventilated cowsheds have become more common. These practices have reduced exposure to gases, dusts and microbes in cowsheds [14]. Dairy farming and forestry are the most important fields of production in the Northern Savo area of Eastern Finland. In 2002 there were 75 400 active farms in Finland. The proportion of dairy farms was 27% in the whole country and 45% in Northern Savo. In France, dairy farms are now most widely developed in the Doubs district in eastern France (3,128 farms with 16,860 active farmers in 2002). Only a few farms still conduct polycultural farming in this area. In the east of France, hay handling is the main source of exposure to microorganisms. The use of silage is forbidden in this region due to quality requirements for the production of Comté cheese.

Microbiological and immunological data in previous studies strongly suggest that *Absidia corymbifera*, *Eurotium amstelodami*, and to a lesser degree *Wallemia sebi*, are involved in FLD [18, 19]. In France, antibodies against *A. corymbifera*, *E. amstelodami* and *W. sebi* are more common in patients with FLD than in control subjects [18]. These results are in accordance with those of a study performed in Finland in which the authors found a level of IgG against *A. corymbifera* three times higher in farmers with FLD than in exposed control farmers [6]. Comparable results have also been found with *W. sebi* and *Eurotium herbariorum*, such as, among the latter, *E. amstelodami* [9, 12].

The aim of the present study was to better understand how climatic factors and agricultural practices influence fungi and actinomycetes concentrations in stored feed. Thus, we compared the microbiological composition of hay, flour and silage samples from different farms chosen to represent the harvest and storage techniques typically used in the regions of Kuopio (Finland) and Doubs (France). Special attention was paid to the presence of the three moulds known to be etiological agents of the disease in both regions.

MATERIALS AND METHODS

Selection of regions. Our study concerned the regions of Kuopio (Finland) and the Doubs (France), 2 dairy farming areas with high prevalence of FLD, 1.7% and 2%, respectively.

Geographic and climatological data. Kuopio is situated at 63º north latitude in the centre of Finland, at an average altitude of 100 m. There is a continental type climate and an average annual temperature of +5ºC. The average annual rainfall is 700 mm.

The Doubs is situated at 47º north latitude in eastern France, at an altitude ranging from 200-1,400 m. There is a continental type climate and an average annual temperature of +7ºC.The average annual rainfall is 800 mm on the plateau and 1,400 mm in the mountains.

Farms. Twenty-three farms - 10 in Kuopio and 13 in the Doubs - were selected on the basis of agricultural production and the different harvest and storage techniques used, including those seldom used. A standardized questionnaire was used to collect background data concerning geographic location of the farm, length of the indoor feeding season, size of the farm and livestock, methods of harvesting and storage of hay, working conditions and length of the indoor feeding season.

Sampling. The sampling was carried out simultaneously in France and in Finland in February 2002. The amounts of microorganisms in hay were expected to be high at this time [19]. Batches of hay were defined as a set of bales or loose hay collected from a group of homogeneous fields over a period of 1-2 days and stored using the same procedure. Samples (n=107) came from the 2001 summer harvest. The first crop of hay (n=36) from Doubs was harvested in May and June and the second (n=27) in July. In Kuopio, a single crop of dry hay (n=29) was harvested in July, ensilage (n=9) was harvested in July, barley was harvested in August and oat in September. Flour samples (n=6) were taken after grain was ground, then placed in sterile bags. The total number of samples per farm ranged from 2-10 (4.6 on average for 23 farms) depending to the material and number of batches available.

Microbiological analyses. Microbiological analyses were carried out according to the methods described previously [18]. After freezing for at least 24 hours to kill mites, samples were cultured on five culture media in adequate temperatures Dichloran-Glycerol (Oxoid, Unipath, Basingstoke, UK) with 0.5% chloramphenicol (Merck, Darmstadt, Germany) at 30ºC for mesophilic mold isolation, 3% malt-agar (Oxoid, Unipath, Basingstoke, UK) with 10% salt and 0.5% chloramphenicol, at 20ºC for osmophilic fungal species, actinomycete isolation agar Bacto medium (Difco, Detroit, MI, USA) at 30ºC for mesophilic actinomycetes and at 52ºC for thermophilic actinomycetes, R8 medium at 52ºC [1]. The number of microbial colonies was counted and the microbes identified after 3 and 7 days. Concentrations of fungi and mesophilic and thermophilic actinomycetes are expressed as colony forming units per gram ($cfu.g^{-1}$) of hay, ensilage or flour. The detection limit was 200 $cfu.g^{-1}$.

Microbiological classification of hay. To estimate the differences in concentration of the 3 moulds involved in FLD (*A. corymbifera*, *Eurotium* spp. and *W. sebi*), we classified the materials on the basis of concentrations into 3 categories: absent, low concentration and high concentration. The highest limit in this classification is the mean concentrations of each fungus given in a previous farm study [20]. The classes for each fungus are established as follows: for *A. corymbifera*, <200 $cfu.g^{-1}$, 200-1,000 $cfu.g^{-1}$ and >1,000 $cfu.g^{-1}$; for *Eurotium* spp., <200 $cfu.g^{-1}$, 200-18,000 $cfu.g^{-1}$ and >18,000 $cfu.g^{-1}$; and for *W. sebi*, <200 $cfu.g^{-1}$; 200-97,000 $cfu.g^{-1}$ and >97,000 $cfu.g^{-1}$.

Statistical analyses. A Kruskal-Wallis test was used to show the influence of haymaking methods on concentration of *A. corymbifera*, *Eurotium* spp. and *W. sebi*. This is a non-parametric test used to test the hypothesis that several samples are from the same population. We used Stata Statistical Sofware: Release 9 (Statacorp. 2005, College station, Texas, USA).

RESULTS

1. Agricultural practices

General data. Milk production per cow was 10,000 kg of milk per year in Kuopio compared with 6,500 kg per year in the Doubs.

In Finland, Holstein-Friesian cows are given fodder consisting mainly of silage and flour (mixture of barley and oat). Hay feeding was only 2 kg per cow per day in Kuopio. In France, the daily amount of hay given to Montbéliarde cows, on average, was 9.3 kg/cow. Flour is seldom given, only in mixed feed.

Description of farms studied. Description of the 23 farms from both regions and the main characteristics of their agricultural practices are summarized in Table 1.

Table 2. weather conditions during the haymaking season in Kuopio (July, 2001) and in the Doubs (May, June and July, 2001).

	Kuopio	Doubs
Number of days without rain (days)	17	9
Total sunshine (hours)	130	97
Precipitation (mm)	98[a]	70
Average daylight (min)	1,200	990

[a]including a rainstorm: 28 mm of water

The average altitude of farms was 105 m in Kuopio and 754 m in Doubs. The mean area and amount of livestock on dairy farms in Kuopio and in Doubs were 33 ha vs. 79 ha, and 32 cattle vs. 99, respectively. In Kuopio dairy farms, the average quantity of hay harvested was 13 tons (3-26 tons per year), the average daily distribution of hay was 67 kg and average work-time in the cowshed 5.9 hours per day. In farms from Doubs, the same average variables were 185 tons of hay (100-323 tons per year), 916 kg per day and 4.9 hours in the cowshed per day, respectively. Three of the farms studied raised horses, 1 in France, 2 in Finland and 1 raised ewes in France.

Hay was left to dry in the field, on average for 7 days in Kuopio vs. 3 days in the Doubs. In Kuopio, it was packed

Table 1. Finnish (K1–K 10) and French (D1-D13) farming conditions and procedures.

N°	Altitude (m)	Total area (ha)	Livestock	Hay making method		Mode of storage[c] (weight in kg)	Tones of hay	Daily hay distribution (kg)	Time in cowshed (h/day)
				In the field[a]	In the barn[b]				
K 1	100	19	3 horses	DF2	WP	RB 400	7	25	0.5
K 2	100	50	35 cows	DF1	T	SP 12	7	25	5
K 3	100	24	59 cows	DF2	WP	RB 400	14	300	6
K 4	100	32	38 cows	DF2	WP	RB 400	5	20	8
K 5	100	16	17 cows	DS	S	Loose	3	10	2
K 6	100	40	30 cows	DF2	WP	RB 400	11	75	8
K 7	150	37	20 cows	DF2	WP	SP 10	26	50	6
K 8	100	43	38 cows	DF1	T	SP 8	11	35	5
K 9	100	24	20 cows	DF1	T	SP 7	26	20	7
K 10	100	13	17 horses	DF1	D	Loose	50	120	5
D 1	900	39	60 cows	DF1	WP	SB 400	124	800	7.5
D 2	500	77	150 cows	DF1	WP	RB 220	155	1,300	5.5
D 3	1,150	112	125 cows	DF1	D	Loose	223	1,500	4
D 4	500	182	220 cows	DF2	WP	RB 220	323	1,200	4.25
D 5	750	47	90 cows	DF1	D	Loose	210	1,000	7
D 6	750	56	40 cows	DF1	D	Loose	194	500	8
D 7	800	44	50 cows	DF1	WP	Loose	145	300	1.75
D 8	1,000	58	75 cows	DF1	D	Loose	195	800	3.5
D 9	900	61	65 cows	DF1	WP	SP 30	132	880	3
D 10	1,000	104	95 cows	DF1	WP	RB 220	238	1,000	4.75
D 11	250	92	120 cows	DF2	WP	RB 250	100	800	4.5
D 12	200	25	25 horses	DF1	WP	SB 400	140	1,000	8
D 13	1,100	4	4 ewes	DF2	WP	Loose	2	15	0.5

[a]Treatment of hay in the field: DF1 = 2-3 days of drying before baling, DF2 = 4-7 days of drying before baling, DS = 14 days of drying in the field in stacks. [b]Treatment of hay in the barn, just after the gathering process: WP = storage with no particular drying processing, D = using of a drier without heating, T = 3-14 days in a 20-bale ventilated drying tunnel without heating, S = salted and stocked on wooden floor. [c]SB = high density square bales; RB = round bales; SP = low-density square bales (small square bales); Loose.

in square low-density bales (7-12 kg) on 4 farms, in round high-density bales (400 kg) on 4 farms, or loose on 2 farms. In the Doubs, hay was harvested loose on 6 farms and in round bales of up to 220 kg on 4 farms. Low- and high-density square bales were used on 1 and 2 farms, respectively. In Kuopio, drying in the field was completed by cool air-drying for low-density square bales and loose hay. In Doubs, ventilation was used only for loose hay.

Meteorological data. Paradoxically, the higher latitude of Kuopio did not create colder or moister conditions. The weather during the haymaking season in 2001 was more favourable in Kuopio than in the Doubs (Tab. 2).

2. Microbiological analyses of hay, ensilage and flour

The differences in frequency and concentrations of isolated species were clearly established. In hay samples, *Eurotium* spp. and *Wallemia sebi* were the most prevalent fungi in both regions. In Finland, the 5 other moulds present in more than 1% of total fungal count were *Aspergillus versicolor*, *Penicillium* spp., *Rhodotorula* sp., *Cladosporium* spp. and *A. corymbifera*. In France, the 2 moulds (in addition to *Eurotium* spp. and *W. sebi*) present in more than 1% of total fungal count were *Cladosporium* spp. and *A. corymbifera*. Means for the concentrations and frequency of microorganisms in hay are presented in Table 3. Both in Finland and in France, isolated actinomycetes, included both mesophilic and thermophilic species (*Streptomyces griseoflavus* and *Saccharomonospora viridis*). *Saccharopolyspora rectivirgula* was only rarely found in material samples from both regions. *Thermoactinomyces vulgaris* was found more in Finland than in France.

Flour samples from the Kuopio region contained mostly *Cladosporium* spp., *Aureobasidium pullulans* and some yeasts. Less than a third of flours contained actinomycetes.

The silage samples contained almost exclusively high quantities of yeasts (>450,000 cfu.g^{-1} in average).

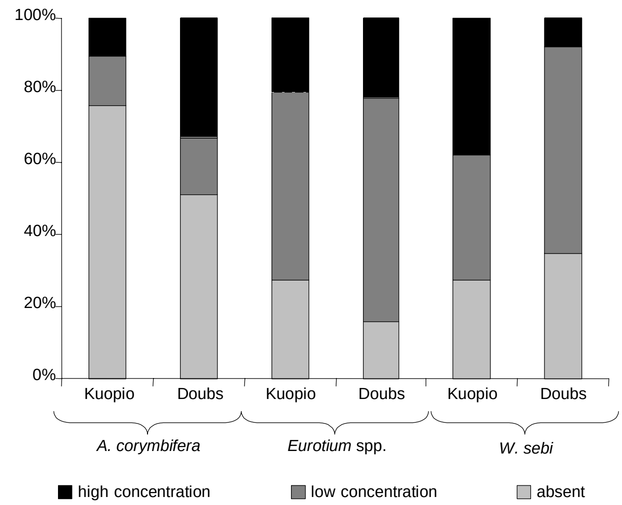

Figure 1. Distribution of *A. corymbifera*, *Eurotium* spp. and *W. sebi* in hay at 3 levels of concentration (absent, low, high concentration) in the 2 areas.

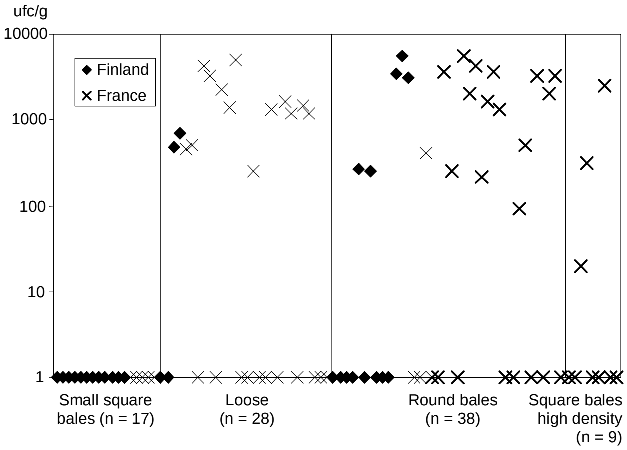

Figure 2. Concentrations of *A. corymbifera* in hay from Finland (n=29) and from France (n=63) according to haymaking method.

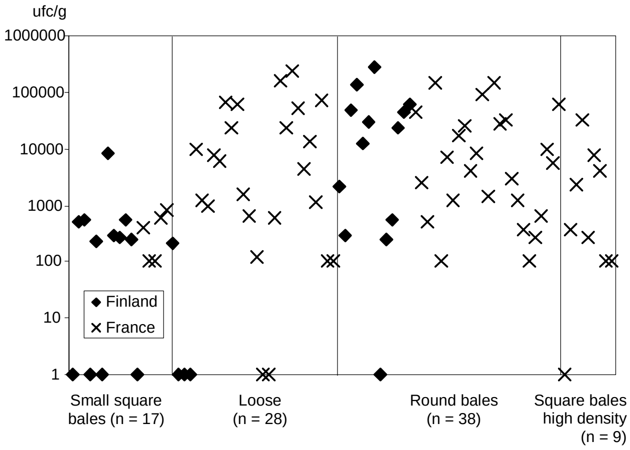

Figure 3. Concentrations of *Eurotium* spp. in hay from Finland (n=29) and from France (n=63) according to haymaking method.

3. Distribution of hay samples into three classes based on the concentrations of *A. corymbifera*, *Eurotium* spp. and *W. sebi*

High concentrations of *A. corymbifera* were found in approximately 35% of Doubs hay samples, but in only 10% of Kuopio hay samples. Conversely, *Eurotium* spp., distribution was more homogeneous, with high concentrations in 20% of hay samples in both regions. High concentrations of *W. sebi* were more typical of Kuopio hay (38% of the samples) than of Doubs hay (8% of the samples) (Fig. 1).

4. Influence of haymaking method on mould exposure

Haymaking methods had an influence on the concentrations of 2 of the 3 moulds involved in FLD: differences were significant for *A. corymbifera* (p=0.007), *Eurotium* spp. (p=0.0005) but not for *W. sebi* (p=0.24). The concentrations of the 3 moulds are presented in Figures 2, 3 and 4, according to the method used in both geographical areas. The results for high-density square bales are shown, despite the fact that this method is used only in Doubs.

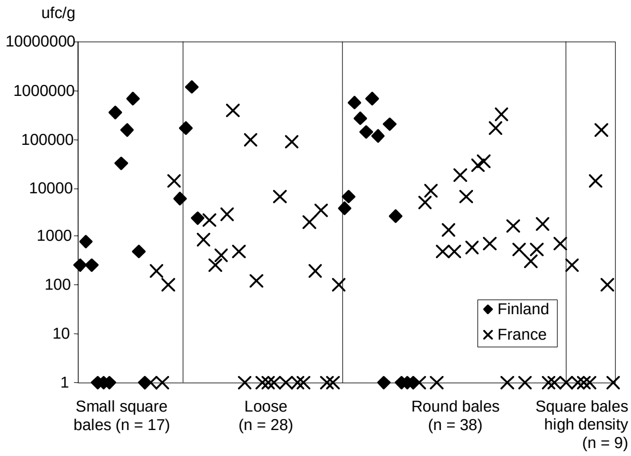

Figure 4. Concentrations of *W. sebi* in hay from Finland (n=29) and from France (n=63) according to haymaking method.

The concentration of *A. corymbifera* was smaller than the detection limit (200 cfu.g^{-1}) in low-density square bales. However, it exceeded 1,000 cfu.g^{-1} in every third sample of both loose hay and round bales. The distribution of concentrations for *Eurotium* spp. and *W. sebi* were rather uniform, irrespective of the haymaking method used. Concentrations of *Eurotium* spp. were found to be lower, however, in low-density square bales than in other hay samples.

DISCUSSION

Meteorological conditions during the 2001 haymaking season differed little between Kuopio (Finland) and the Doubs (France). The total amount of sunshine, duration of daylight and number of days without rain, however, were more favourable in Kuopio than in the Doubs, located at a

Table 3. Means and frequencies of microorganisms isolated in hay, flour and silage in Finland and in France.

	Doubs hay		Kuopio hay		Kuopio silage		Kuopio flour	
	Mean (SD)	Prevalence of fungi (%)	Mean (SD)	Prevalence of fungi (%)	Mean (SD)	Prevalence of fungi (%)	Mean (SD)	Prevalence of fungi (%)
Fungi								
Wallemia sebi	22,000 (69,500)	65	157,100 (282,700)	72	-	-	-	-
Eurotium spp.[a]	22,800 (45,800)	95	22, 600 (57,700)	72	-	-	900 (1,700)	50
Aspergillus versicolor	20 (100)	6	12,100 (45,500)	10	-	-	-	-
Penicillium spp.	30 (120)	8	11,000 (45,300)	24	60 (170)	11	340 (450)	50
Cladosporium spp.	3,900 (21,100)	29	400 (800)	59	-	-	8,600 (9,100)	100
Absidia corymbifera	900 (1,400)	49	400 (1,300)	24	-	-	330 (820)	17
Aspergillus spp.[b]	230 (940)	27	180 (450)	28	520 (1,570)	11	70 (110)	33
Other fungi[c]	280 (690)	30	260 (940)	24	-	-	5,170 (5,830)	100
Yeasts								
Rhodotorula spp.	-	-	1,200 (4,100)	31	-	-	6,800 (5,000)	100
Geotrichum capitatum	-	-	-	-	138,900 (416,700)	11	-	-
Other yeasts	16,200 (125,900)	14	8,500 (45,500)	17	330,100 (828,000)	33	7,700 (8 200)	83
Actinomycetes								
Mesophilic *Streptomyces*	6,500 (14,900)	57	37,900 (169,200)	45	80 (180)	22	230 (440)	33
Thermophilic *Streptomyces*	3,600 (9,300)	97	2,600 (6,100)	62	830 (2,500)	11	1,870 (4,500)	33
Thermoactinomyces vulgaris	150 (1,120)	5	270 (520)	41	890 (2,500)	33	112 (270)	17
Saccharomospora viridis	700 (2,600)	33	1,300 (4,300)	31	60 (170)	11	-	-
Saccharopolyspora rectivirgula	13 (90)	5	9 (50)	3	30 (90)	11	770 (1,780)	33

SD: standard deviation; -: not found; [a]including *E. amstelodami*, *E. herbariorum* ; [b]*Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus candidus*, *Aspergillus flavus* ; [c]*Alternaria* spp., *Fusarium* spp., *Aureobasidium pullulans*, *Acremonium* spp., *Mucor* spp. and sterile mycelium lower latitude but in a mountainous region. As this may have been an exceptional summer, a follow-up study covering several successive years should be conducted.

The main difference between the 2 regions is cow fodder. In Kuopio, the average quantity of milk yield per cow is high and most of the daily fodder consists of silage and flour, and only a small amount of hay. In the Doubs, fodder consists mainly of hay and the amount of flour used is limited. It is forbidden to use silage in the area where Comté cheese is produced.

Although production and quantities of hay handled differ radically between the 2 regions, daily exposure time in the cowshed (5.9 h/day vs. 4.9 h/day) is nearly the same. Farmers from the Doubs handle on average 3 time more hay than those from Kuopio. On the other hand, the latter handle more flour than their French counterparts. This difference in feeding practices has a considerable effect on the work-related risk of contracting respiratory disorders.

Feeding material affects the quality and the quantity of bioaerosol exposure arising in the cowshed during feeding. The microbiological composition of flour, mainly yeasts with wet walls, represents a lower risk for air contamination than that of hay, which is the main source of inhalable microorganisms. The microbiological composition of hay differs in the 2 regions: *Rhodotorula* spp. was frequently isolated in samples from Kuopio (9/29), but not in those from Doubs. Conversely, *A. corymbifera* was isolated more frequently in hay samples from the Doubs (31/63) than in those from Kuopio (7/29). The concentration of *A. corymbifera* in hay samples from the Doubs was twice that of those from Kuopio. These differences most probably have an effect on farmers' exposure.

The role of *Saccharopolyspora rectivirgula* as an etiologic agent of FLD, was recently re-evaluated in both areas. Immunological reaction determined by FLD patients with this antigen was low and not clearly related with FLD cases either in France or in Finland [13, 18]. Our present study confirms that today *S. rectivirgula* is seldom present in hay, flour or silage in either region. This evolution could signify that the prevention campaigns carried out in both regions have contributed to changes in agricultural practices that prevent conditions humid enough for the growth of *S. rectivirgula* [8].

Agricultural practices have an effect on the nature of bioaerosols and on the incidence of FLD [15]. Methods of cutting, windrowing, harvesting and storing hay are crucial factors that affect the microbiological quality of hay. It is obvious that technical developments in agricultural practice have modified microbial exposure on farms. It is currently quite unusual to dry hay in stacks, a haymaking method which allowed Pépys *et al.* to isolate up to 85% of *S. rectivirgula* [7, 16].

In our study, *A. corymbifera* seems to be one of the main thermophilic species isolated in hay. Unfortunately, its spores have the size and antigenic compounds required to be an etiologic agent of FLD. *A. corymbifera* was not isolated in low-density square bales, either in Finland or in France. This method of making hay is disappearing in Doubs because of the high cost and workforce required. It is being replaced by round bales, which are easier both to store and to handle mechanically. The disadvantage of round bales is their high humidity content, probably due to their higher density, which favours the proliferation of thermophilic species. Warm air-drying of loose hay is an alternative way to improve the microbiological quality of hay [2]. In our study, loose hay was less contaminated by *A. corymbifera* than round bales of hay (53% and 63% of positive samples, respectively). To prevent moulding, simple, new economical technologies should be developed to obtain better quality hay during a rainy harvest season.

Serological tests have been used to confirm the microbial exposure that led to FLD. The selection of antigens used in these antibody tests should reflect the most probable microbial exposures occurring on farms. Differences in the microbiological quality of hay and regional differences in microbial exposures have to be taken into account in choosing antigens for serological tests in order to increase the sensitivity of the test. If present, sources of microbes other than hay, e.g. straw and peat used as bedding materials [11], should be considered in relevant antigen selection.

Concerning primary prevention, screening and serological diagnosis, it is strongly recommended to follow developments of agricultural practices.

Work practices have a strong effect on microbial exposures on farms, and therefore also on the potential health risks. The quality of hay is influenced mainly by meteorological conditions [19, 23]. The moisture in newly-stored hay is well-known for affecting the developing of fungi and actinomycetes [8]. Another factor, which has received less attention, is the windrowing and the possible mixture of earth containing microbes [19] with hay. This may lead to high microbial concentrations in the hay handled, with no visible mould.

Further studies are needed to determine agricultural practices associated with minor risks involved in the development of harmful microorganisms. This approach would differ from those that add chemicals to limit the development of microorganisms in hay [10, 17], or those that inoculate hay with a microbe suspension without sufficient knowledge as to its impact on human health [4, 5].

Our results reflect one harvest season; several years of follow-up are needed to draw firmer conclusions. More detailed record-keeping on each farm would help to evaluate the effect of other possible factors on the results. However, it is important to note that despite different concentration, only minor differences were found in possible exposure to FLD inducing microbes. In addition to microbiological analyses, future studies should include questionnaires on symptoms and serological studies in farmers in order to learn more about the links between agricultural practices, microbial exposure, immunological response and respiratory symptoms. Collaborative European studies on work-related risks in agriculture are needed, especially to promote respiratory health in a diminishing farming population.

Acknowledgements

This work was supported by the French research team "Santé et Environnement Rural, Université de Franche-Comté" (SERF EA 2276) and the city of Besançon.

The authors are grateful to Nancy Richardson-Peuteuil for her editorial assistance, to Dr Terttu Vilponen-Salmela for her help during the journey at Kuopio, and Marie-Françoise Poitout from the "Franco-Finlandaise" association.

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