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IgG antibodies, chronic bronchitis, and pulmonary function values in farmer's lung patients and matched controls

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Key words: chronic bronchitis; farmer's lung; IgG antibodies; respiratory function tests; serology.

We measured IgG antibody levels against eight different microbes in farmer's lung (FL) patients an average of 14 years after the first diagnosed episode of FL and in matched controls. The study population consisted of 87 FL patients and 81 control farmers, matched by age, sex, and smoking habits. Clinical studies included the measurement of IgG antibody levels against *Absidia corymbifera*, *Aspergillus umbrosus*, *A. fumigatus*, *Humicola grisea*, *Saccharopolyspora rectivirgula*, *Penicillium brevicompactum*, *Rhodotorula glutinis*, and *Thermoactinomyces vulgaris*, in addition to spirometry, pulmonary diffusing capacity (DL_{CO}), and the evaluation of chronic bronchitis. Median IgG antibody levels were two or more times higher in FL patients than control farmers against *Ab. corymbifera*, *S. rectivirgula*, and *T. vulgaris* ($P < 0.001$). Against *A. fumigatus*, *H. grisea*, and *R. glutinis*, FL patients also had significantly higher antibody levels. FL patients often had positive antibody titers against several microbes, whereas control farmers usually had a positive titer against one or two microbes. A positive association between IgG antibody levels and chronic bronchitis and DL_{CO} was observed in FL patients, but not in control farmers. It is suggested that the high antibody levels noted in FL patients were due not only to high exposure but also to individual sensitivity to environmental microbes.

Date:

Accepted for publication 16 June 1999

To cite this article:

Erkinjuntti-Pekkanen R., Reiman M., Kokkarinen J.I., Tukiainen H.O. & Terho E.O. IgG antibodies, chronic bronchitis, and pulmonary function values in farmer's lung patients and matched controls. *Allergy* 1999; **54**: 1181–1187.

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ISSN 0105-4538

It has been shown that the great majority of farmer's lung (FL) patients, as well as many "healthy" farmers, have serum IgG antibodies against thermophilic actinomycetes or fungal antigens (1, 2). The presence of these antibodies is thought to be an evidence of exposure, but their exact role in the pathogenesis and long-term outcome of FL is still unclear (3–6).

As the microbiologic flora of moldy hay varies widely in different countries, the antigens for serologic studies should be selected on the basis of determination of local species (7). In previous studies from Finland, the highest rates of IgG antibodies in FL patients have been found against *Thermoactinomyces vulgaris* and *Aspergillus* species, and lower rates against *Saccharopolyspora rectivirgula* (2). Only those "traditional" antigens mentioned above have been measured in previous studies. Later studies have shown that *Absidia corymbifera*, *Humicola grisea*, *Penicillium brevicompactum*, and *Rhodotorula glutinis* occur frequently in Finnish farms, in hay, grain, and straw (8, 9). Therefore, it has been suggested that these microbes are at least as important as the "traditional" FL-related antigens in terms of exposure in farming environments (9). However, there is very little knowledge, if any, about the IgG antibody levels against these "new" microbes.

The aims of this study were as follows:

- 1) to determine the IgG antibody levels against four "traditional" FL-related microbes, *Aspergillus umbrosus*, *A. fumigatus*, *S. rectivirgula*, and *T. vulgaris*, and against four "new" microbes, *Ab. corymbifera*, *H. grisea*, *P. brevicompactum*, and *R. glutinis*, in FL patients at a mean of 14 years after the diagnosis of FL and in matched control farmers
- 2) to investigate whether there is any association between the antibody levels and chronic bronchitis or pulmonary function status.

Material and methods

Study population

Of the 101 FL patients included in a previous study on FL (10), those 97 still living were asked to participate in the present study. In the four patients who had died, the causes of death were unrelated to FL. Six patients refused to take part in clinical investigations, and two patients were excluded due to lack of a suitable control farmer. From two patients the serum samples were excluded from the analysis because they had had a recurrent episode of FL during the preceding 2 years. Thus, the present analyses include 87 FL patients. The pulmonary function data and HRCT findings of the same study population have been reported earlier (11, 12).

All FL patients were diagnosed at Kuopio University Hospital in a dairy farming area in eastern Finland in 1977–82. For all the patients, this was their first diagnosed episode of FL, and all fulfilled the diagnostic criteria for FL presented by Terho (13). Fifty-three patients had experienced only one single acute episode of FL. Thirty-four (39%) patients had had recurrent episodes of FL; 25 had had one recurrence, seven had had two, one had had three, and one had had four recurrences. The interval between the last recurrence and the present study was, on average, 8 years.

Control farmers were selected from 2424 participants in a questionnaire survey assessing farmers' health carried out in 1979 (14), to which all farmers from three municipalities in the Kuopio University Hospital district were invited. More than 94% of those invited had participated in the study in 1979. The three municipalities are geographically and socioeconomically representative of the whole Kuopio University Hospital district. Since all FL patients had cattle tending as their main farm work, only control farmers with the same occupation in 1979 were selected. FL patients and control farmers were divided into strata on the basis of age (5-year age categories), sex, and smoking habits at the time of the diagnosis or in the 1979 survey (lifetime nonsmoker, ex-smoker, or current smoker). Within each stratum, four times more control farmers were randomly selected than there were FL patients in that stratum.

One control for each FL patient was invited to a clinical examination. If a control farmer was unable to attend, a new control farmer from the same stratum was selected. Eighty-six of the 138 invited control farmers (62%) participated in the clinical examinations. Two control farmers were excluded from the analyses, one because her smoking habit was wrongly coded in the initial study in 1979, and one because she herself had suffered from FL 3 years earlier. Three control farmers did not give a serum sample. Thus, 81 control farmers were included in the present analyses.

Clinical examinations, which consisted of pulmonary function measurements, high-resolution CT, and determination of IgG antibody levels, took place from August 1993 to April 1994. When the present study was performed, there had been a mean interval of 14 years (range 11–16 years) after the initial diagnosis of FL disease. The clinical data of the study population are shown in Table 1.

Serologic testing

All serum samples were first stored at -20°C for up to 2 weeks, and then at -70°C until analyzed. IgG antibody levels in serum samples were determined against antigens

from the mesophilic fungi *Aspergillus (A.) umbrosus* (strain M118), *Penicillium (P.) brevicompactum* (strain M129), and *Rhodotorula (R.) glutinis* (strain MY112); the thermo-tolerant fungi *Absidia (Ab.) corymbifera* (strain M138), *Aspergillus (A.) fumigatus* (strain M150), and *Humicola (H.) grisea* (strain M149); and the thermophilic actinomyces *Saccharopolyspora rectivirgula* (formerly called *Micropolyspora faeni*) (strain A111) and *Thermoactinomyces (T.) vulgaris* (strain A105). The method of antigen preparation has been described earlier in detail (15). The IgG antibody concentrations were determined by ELISA, with the mycelial sonicates as antigens (15). All measurements were conducted at the Kuopio Regional Institute of Occupational Health.

Antibody response scoring

Classified titers

Absorbance values were classified from titer 0 to titer 4. Absorbance values at least two standard deviations higher than the mean absorbance in a healthy nonrural control group were classified as titer 1. Absorbance values at least four standard deviations higher than the mean absorbance in the healthy control group were classified as titer 2.

Absorbance values at least six standard deviations higher than the mean absorbance in the healthy control group were classified as titer 3. Titers 2 and 3 were considered positive. The term "titer" is used in the results to refer to classified titers. *Total antibody score* is the sum of the antibody titers against eight different microbes.

Pulmonary function tests

All pulmonary function measurements were conducted by the same trained nurse. Spirometry (SensorMedics 2200, Yorba Linda, CA, USA) was performed according to ATS guidelines (16). The reference values were according to Viljanen et al. (17). Measurement of pulmonary diffusing capacity (DL_{CO}) (SensorMedics 2200, Yorba Linda, CA, USA) was conducted with a single-breath technique (18) with the reference values according to the ECSC (19).

Respondents were classified as having chronic bronchitis if they had coughed up phlegm on most days for at least 3 months per year for at least 2 years. Subjects who fulfilled the criteria of chronic bronchitis but also had asthma were excluded from the chronic bronchitis group.

The study protocol was approved by the ethics committee of Kuopio University Hospital.

Statistical analyses

The statistical significance of differences between means was tested by analyses of covariance with SPSS statistical packages. All comparisons between the study groups were adjusted for sex, smoking at the time of diagnosis or in 1979 in three categories (nonsmoker, ex-smoker, current smoker), and age as a continuous variable. All analyses of pulmonary function also included height as a continuous variable. The mean pulmonary function values presented in the tables have been adjusted for age, sex, height, and smoking habits by analysis of covariance (SAS statistical package).

Results

At the time of the follow-up, 76% of the FL patients and 86% of the control farmers were still living on a farm, 39% of the FL patients still worked regularly in a cow house, 4% worked occasionally, and 57% did not work on a farm. The respective figures for control farmers were 38%, 7%, and 55%. Fifty-five percent of the FL patients but only 12% of the control farmers had used a half-mask-type or powered dust helmet respirator, and 24% of the FL patients and 18%

Table 1. Clinical data of FL patients and control farmers

	FL patients		Control farmers	
	<i>n</i>	(%)	<i>n</i>	(%)
All	87		81	
Women	67	(77)	61	(75)
Nonsmokers	73	(84)	68	(84)
Retired	60	(69)	53	(65)
	Mean	(SD)	Mean	(SD)
Age, years	60	(9)	60	(9)
(minimum, maximum)		(38–76)		(40–76)
<i>Beginning of follow-up</i>				
Area under cultivation, acres	16	(9)	14	(7)*
Dairy cattle, no. of animals	11	(6)	9	(4)*
<i>End of follow-up</i>				
Area under cultivation, acres	16	(13)	14	(13)*
Dairy cattle, no. of animals	13	(7)	12	(6)

**P* < 0.05.

of the control farmers had used a disposable paper mask only while working on a farm.

FL patients had higher serum IgG antibody levels against all measured microbes than the control farmers (Table 2). Seventy-eight percent of the FL patients and 54% of the control farmers had a positive IgG antibody titer against at least one of the eight microbes (Table 3). The biggest differences between the study groups were observed in the antibody levels and positive antibody titers of *S. rectivirgula*, *Ab. corymbifera*, *T. vulgaris*, and *R. glutinis*. *A. fumigatus* and *A. umbrosus* were the most common positive antibody titers in both study groups. There were no significant differences between men and women in the antibody levels against any of the eight different microbes, nor were there differences between the antibody levels of FL patients with single or recurrent episodes of FL.

Both FL patients and control farmers with current antigen exposure (who visited a cowshed occasionally or regularly) had statistically significantly higher antibody levels against *S. rectivirgula* and *T. vulgaris* than farmers without exposure (did not live on a farm, had stopped farming, or did not visit cowshed). In FL patients, the other antibody levels were not associated with current exposure. In control farmers, those with current antigen exposure had a slight tendency toward higher antibody levels also against most of the other microbes (data not shown).

FL patients who had chronic bronchitis had a tendency toward higher antibody levels against all eight microbes than FL patients without chronic bronchitis or control farmers (Table 4). Antibody levels against *Ab. corymbifera*, *P. brevicompactum*, and *R. glutinis* were significantly higher among FL patients with chronic bronchitis than FL patients without chronic bronchitis. Furthermore, 36% ($n=15$) of

Table 3. Prevalence of positive IgG antibody titers* in sera of FL patients and control farmers

	FL patients $n=87$ %	Control farmers $n=81$ %	P^{**}
<i>Ab. corymbifera</i>	20	4	0.004
<i>A. fumigatus</i>	51	41	0.2
<i>A. umbrosus</i>	45	32	0.08
<i>H. grisea</i>	1	0	0.7
<i>S. rectivirgula</i>	39	10	<0.001
<i>P. brevicompactum</i>	18	12	0.3
<i>R. glutinis</i>	39	21	0.01
<i>T. vulgaris</i>	21	5	0.005
Any one of eight	78	54	0.004

*Absorbance values of at least four standard deviations higher than mean absorbance in healthy control group were considered positive.
**Adjusted for age, sex, and smoking.

those FL patients with total antibody scores of ≥ 8 ($n=42$) had chronic bronchitis, as compared to only 16% ($n=7$) of those FL patients with total antibody scores of 0–7 ($n=45$) ($P=0.03$). Among control farmers, there was no association between chronic bronchitis and IgG antibody levels.

Total antibody scores showed a significant inverse association with mean DL_{CO} values in FL patients (Table 5); those FL patients who had antibody scores of ≥ 12 had DL_{CO} an average of 11% lower than those with antibody scores of 0–3. A similar but weaker trend was also observed in control farmers. In FL patients, mean vital capacity (VC) and FEV_1 values were lowest in those with the highest antibody scores (Table 5). In control farmers, no association between antibody scores and mean VC and FEV_1 values was noted. We also analyzed the association between

Table 2. Median IgG antibody levels* against eight different microbes in sera of FL patients and control farmers

	FL patients $n=87$		Control farmers $n=81$		P^{**}
	Median	(minimum, maximum)	Median	(minimum, maximum)	
<i>Ab. corymbifera</i>	433	(34, 2058)	155	(31, 1980)	<0.001
<i>A. fumigatus</i>	794	(75, 2591)	625	(98, 2206)	0.01
<i>A. umbrosus</i>	419	(56, 1910)	267	(28, 1481)	0.08
<i>H. grisea</i>	244	(38, 888)	172	(31, 672)	0.01
<i>S. rectivirgula</i>	404	(70, 2163)	199	(41, 1571)	<0.001
<i>P. brevicompactum</i>	269	(35, 2066)	189	(25, 1709)	0.1
<i>R. glutinis</i>	1450	(370, 2066)	1006	(227, 2599)	<0.001
<i>T. vulgaris</i>	202	(29, 1041)	101	(27, 539)	<0.001

*Absorbance at 405 nm.
**Adjusted for age, sex, and smoking.

Table 4. Median antibody levels* against different microbes in FL patients and control farmers with or without chronic bronchitis

	FL patients Chronic bronchitis			Control farmers Chronic bronchitis		
	Present n = 16 Median	Not present n = 71 Median	P**	Present n = 14 Median	Not present n = 67 Median	P**
<i>Ab. corymbifera</i>	564	387	0.02	156	154	0.5
<i>A. fumigatus</i>	1093	677	0.07	440	631	0.97
<i>A. umbrus</i>	469	385	0.4	144	296	0.8
<i>H. grisea</i>	309	215	0.08	168	179	0.6
<i>S. rectivirgula</i>	478	391	0.3	195	220	0.3
<i>P. brevicompactum</i>	515	215	0.04	198	185	0.6
<i>R. glutinis</i>	1665	1288	0.03	1098	961	0.9
<i>T. vulgaris</i>	230	201	0.3	84	106	0.2

*Absorbance at 405 nm.
**Adjusted for age, sex, and smoking habits.

pulmonary function values and different antibody levels separately in FL patients and control farmers, dividing subjects into quartiles of different antibody levels (data not shown). In general, FL patients who had the highest antibody levels against different microbes tended to have the lowest mean VC, FEV₁, and DL_{CO} values, and a statistically significant ($P=0.004$) negative association between antibodies against *Ab. corymbifera* and DL_{CO} was noted. In control farmers, no similar association was noted.

Table 5. Association between antibody score and pulmonary function* in FL patients and control farmers

FL patients					
Antibody score	0–3 n = 21	4–7 n = 24	8–11 n = 21	≥ 12 n = 21	P**
VC, l	3.86	3.77	3.83	3.63	0.2
FEV ₁ , l/s	2.69	2.57	2.67	2.43	0.1
MEF _{50%} , l/s	2.52	2.58	2.61	2.27	0.5
FEV%	69.6	68.2	70.0	66.7	0.4
DL _{CO} , mmol/min/kPa	8.13	7.98	7.38	7.20	0.03
Control farmers					
Antibody score	0–1 n = 27	2–4 n = 20	5–8 n = 17	≥ 9 n = 17	P**
VC, l	3.63	3.69	3.56	3.67	0.9
FEV ₁ , l/s	2.63	2.72	2.61	2.70	0.9
MEF _{50%} , l/s	3.09	3.29	3.28	3.29	0.7
FEV%	72.5	73.9	73.6	73.7	0.7
DL _{CO} , mmol/min/kPa	8.94	8.80	8.68	8.30	0.3

*Values have been adjusted for age, sex, height, and smoking.
**Test for trend, analyses of covariance, adjusted for age, sex, height, and smoking.

Discussion

In the present study, FL patients who had had their first FL episode a mean 14 years earlier had significantly higher IgG antibody levels against six of the eight measured microbes than control farmers.

It is well known that patients at an acute or subacute stage of FL have higher microbe-specific IgG antibody levels than farmers with no evidence of the disease (20, 21). It has also been shown that these microbe-specific IgG antibodies are a common finding years after the acute stage of FL (22–24). Unfortunately, we did not have the data of the antibody status at the beginning of the follow-up. Although Katila et al. (25) showed in a 6-year follow-up of healthy dairy farmers that individual changes in antibody titers were uncommon, it is not justified to assume that the antibody levels measured in this study represent the start-point exposure.

In our study, positive antibody titers against *S. rectivirgula* were twice as common as against *T. vulgaris* in both study groups. The largest differences between the study groups were observed in the positive antibody titers against *Ab. corymbifera*, *S. rectivirgula*, *T. vulgaris*, and *R. glutinis*. In previous Finnish studies, the highest rates of IgG antibodies have been usually found against *T. vulgaris* and *Aspergillus* species, and lower rates against *S. rectivirgula*, both in patients with FL and in healthy farmers (2, 26). *Ab. corymbifera* and *R. glutinis* have not been included in previous studies. Preliminary results from France have suggested that *Ab. corymbifera* could be one of the main antigens of FL in the Doubs department (27). The

role of *Ab. corymbifera* in acute FL should be studied also in Finland.

The differences in the antibody findings of the present and the previous Finnish studies may be due to antigenic differences in different strains or, most probably, to changes in antigen exposure. Exposure to microbes may have changed since the 1970s and 1980s, when most of the previous Finnish studies were performed. During the last 15 years, great changes have occurred in farming conditions in Finland (28). For example, the use of straw as bedding material has diminished by half, and baling of hay has increased by 60% (28). Straw is known to be the main source of *T. vulgaris*. Baled hay is the main source of *Ab. corymbifera* and *P. brevicompactum* (9, 29).

In our study, FL patients had higher IgG antibody levels than the control farmers, and FL patients with the highest IgG levels had the highest risk of both chronic bronchitis and lowered DL_{CO}. There are two different explanations for the higher antibody levels observed in FL patients than control farmers:

- 1) FL patients had higher exposure to the measured microbes
- 2) FL patients are individually more susceptible to that exposure.

As the work environments were not investigated in this study, we could not assess intergroup differences in the quality and quantity of occupational exposure. However, the total antigen-exposure time was believed to have been similar in both groups. After the matching of the FL patients to the control farmers in 1979, the groups remained very similar with respect to the proportions working on farms and tending cattle as well as the main farm operation. Thus, after 1979, the exposure time would have been similar in both groups. In Finland, farmers have usually been raised on a farm and have worked in farming since their youth. In the present study, the educational levels of the study groups were similar. Therefore, there is no reason to assume that the total exposure time of FL patients would have been very different from that of control farmers.

We do not know whether the FL patients had higher exposure to the measured environmental microbes than the control farmers. A few studies have shown farmers with FL to have been exposed to greater spore concentrations than reference farmers (8, 30), and that may be true also of FL patients in our study at least at the time of their first episode

of FL. However, after their recovery from FL, farmers were advised to avoid moldy material, as well as use personal dust respirators while working on the farm. We believe that most of the farmers with FL took these preventive measures seriously. In our study, 79% of the FL patients compared to 30% of the control farmers used some kind of personal dust respirator.

It is interesting that we found a positive association between the total antibody score and the presence of chronic bronchitis and mean DL_{CO} only in FL patients, and not in control farmers. In previous studies, mostly done among healthy farmers, the results on this association between IgG antibody levels and respiratory symptoms have been very controversial; both negative (20, 23, 31) and positive (32–34) associations have been reported. Most of the previous studies of healthy farmers have found no association between antibody status and pulmonary function (32, 33, 35). However, in a nearly 15-year follow-up, 92 FL patients farmers who had persistently positive findings of antibodies against *S. rectivirgula* and *T. vulgaris* had significantly lower DL_{CO} than those who had become seronegative (23).

If the high IgG level among FL patients had been due only to higher exposure, a factor which could also explain these respiratory findings, there should have been a similar association between IgG levels and chronic bronchitis and DL_{CO} also among control farmers. Thus, it is possible that FL patients are more sensitive to environmental microbes (and among them some are even more sensitive than others), and this is the reason for both high IgG antibody levels and chronic bronchitis, as well as for lowered DL_{CO}.

In conclusion, FL patients 14 years after the diagnosis of FL still had significantly higher IgG antibody levels against microbes common in Finnish farms. The largest differences were observed in the levels of the two “traditional” (*S. rectivirgula* and *T. vulgaris*) and the two “new” (*Ab. corymbifera* and *R. glutinis*) antibodies. The reason for the high IgG antibody levels remains uncertain, but it is possible that these levels were influenced by individual sensitivity to environmental microbes, a factor which is also related to the risk of chronic bronchitis and lowered DL_{CO}.

Acknowledgments This study was supported by grants from the Farmers' Social Insurance Institution of Finland, the Yrjö Jahnsson Foundation, and the Finnish Anti-Tuberculosis Association Foundation.

References

- Pepys J. Immunopathology of allergic lung disease. *Clin Allergy* 1973;**3**:1–22.
- Terho EO, Lacey J. Microbiological and serological studies of farmer's lung in Finland. *Clin Allergy* 1979;**9**:43–52.
- Pepys J. Antigens and hypersensitivity pneumonitis. *J Allergy Clin Immunol* 1978;**61**:201–203.
- Burrell R, Rylander R. A critical review of the role of precipitins in hypersensitivity pneumonitis. *Eur J Respir Dis* 1981;**62**:332–343.
- Terho EO. Extrinsic allergic alveolitis – the state of the art. *Eur J Respir Dis* 1982;**63** Suppl 124:10–26.
- Salvaggio JE. Immune reactions in allergic alveolitis. *Eur Respir J* 1991;**4** Suppl 13:478–598.
- Kurup VP, Mäntyjärvi RA, Terho EO, Ojanen TH, Kalbfleisch JH. Circulating IgG antibodies against fungal and actinomycete antigens in the sera of farmer's lung patients from different countries. *Mycopathologia* 1987;**98**:91–99.
- Kotimaa MH, Husman KH, Terho EO, Mustonen MH. Airborne moulds and actinomycetes in the work environment of farmer's lung patients in Finland. *Scand J Work Environ Health* 1984;**10**:115–119.
- Kotimaa MH. Spore exposure arising from stored hay, grain and straw. *J Agric Soc Finland* 1990;**62**:285–291.
- Kokkarinen JI, Tukiainen HO, Terho EO. Recovery of pulmonary function in farmer's lung. A five-year follow-up study. *Am Rev Respir Dis* 1992;**145**:3–5.
- Erkinjuntti-Pekkanen R, Kokkarinen JI, Tukiainen HO, Pekkanen J, Husman K, Terho EO. Long-term outcome of pulmonary function in farmer's lung: a 14 year follow-up with matched controls. *Eur Respir J* 1997;**10**:2046–2050.
- Erkinjuntti-Pekkanen R, Rytönen H, Kokkarinen JI, Tukiainen HO, Partanen K, Terho EO. Long-term risk of emphysema in patients with farmer's lung and matched control farmers. *Am J Respir Crit Care Med* 1998;**158**:662–665.
- Terho EO. Diagnostic criteria for farmer's lung disease. *Am J Ind Med* 1986;**10**:329.
- Vohlonen I, Husman K, Kalimo E, et al. *Farmers' work and health 1979* [In Finnish with English Abstract]. Helsinki: Publications of the Social Insurance Institution, Finland, A:17, 1981.
- Ripatti T, Koskela P, Kotimaa M, Koskinen E, Mäenpää PH. Serum IgG antibody concentrations against environmental microbes in mares and foals during different seasons and effect of stabling practices. *Am J Vet Res* 1990;**51**:550–555.
- American Thoracic Society. Standardization of spirometry – 1987 update. *Am Rev Respir Dis* 1987;**136**:1285–1298.
- Viljanen AA, Halttunen PK, Kreus K-E, Viljanen BC. Spirometric studies in non-smoking healthy adults. *Scand J Clin Lab Invest* 1982;**42** Suppl 159:5–20.
- American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for standard technique. *Am Rev Respir Dis* 1987;**136**:1299–1307.
- Cotes JE, Chinn DJ, Quanjer PhH, Roca J, Yernault J-C. Standardization of the measurement of transfer factor (diffusing capacity). *Eur J Respir Dis* 1993;**6** Suppl 16:41–52.
- Roberts RC, Wenzel FJ, Emanuel DA. Precipitating antibodies in a Midwest dairy farming population toward the antigens associated with farmer's lung disease. *J Allergy Clin Immunol* 1976;**57**:518–524.
- Katila M-L, Mäntyjärvi RA. The diagnostic value of antibodies to the traditional antigens of farmer's lung in Finland. *Clin Allergy* 1978;**8**:581–587.
- Barbee RA, Callies Q, Dickie HA, Rankin J. The long-term prognosis in farmer's lung. *Am Rev Respir Dis* 1966;**97**:223–231.
- Braun SH, doPico GA, Tsiatis A, Horvath E, Dickie HA, Rankin J. Farmer's lung disease: long-term clinical and physiologic outcome. *Am Rev Respir Dis* 1979;**119**:185–191.
- Cormier Y, Bélanger J. Long-term physiologic outcome after acute farmer's lung. *Chest* 1985;**87**:796–800.
- Katila M-L, Ojanen T, Mäntyjärvi R. A six-year follow-up of antibody levels against microbes present in the farming environment in a group of dairy farmers in Finland. *Am J Ind Med* 1986;**10**:307–309.
- Terho EO, Husman K, Vohlonen I, Mäntyjärvi RA. Serum precipitins against microbes in mouldy hay with respect to age, sex, atopy, and smoking of farmers. *Eur J Respir Dis* 1987;**71** Suppl 152:115–121.
- Reboux G, Madroszyk A, Millon L, et al. *Absidia corymbifera*: a new antigen of farmer's lung? *Am J Respir Crit Care Med* 1999;**159** (Suppl):A300 [Abstract].
- Louhelainen K, Kotimaa M, Taattola K. Pölyvaara ja työmenetelmät rehujen ja kuivikkeiden käsittelyssä. In: Susitaival P, editor. *Farming and occupational health in Finland in 1992* [In Finnish with English Abstract]. Helsinki: Publications of the Social Insurance Institution, Finland, ML:133, 1994:197–212.
- Kotimaa MH, Oksanen L, Koskela P. Feeding and bedding materials as sources of microbial exposure on dairy farms. *Scand J Work Environ Health* 1991;**17**:117–122.
- Malmberg P, Rask-Andersen A. Natural and adaptive immune reactions to inhaled microorganisms in the lungs of farmers. *Scand J Work Environ Health* 1988;**14** Suppl 1:68–71.
- Cormier Y, Bélanger J, Durand P. Factors influencing the development of serum precipitins to farmer's lung antigen in Quebec dairy farmers. *Thorax* 1985;**40**:138–142.
- Guernsey JR, Morgan DP, Marx JJ, Horvath EP, Pierce WE, Merchant JA. Respiratory disease risk relative to farmer's lung disease antibody status. In: Dosman JA, Cockcroft DW, editors. *Principles of health and safety in agriculture*. Boca Raton, FL: CRC Press, 1989:81–84.
- Marx JJ, Guernsey J, Emanuel DA, Merchant JA, Morgan DP, Kryda M. Cohort studies of immunologic disease among Wisconsin dairy farmers. *Am J Ind Med* 1990;**18**:263–268.
- Dalphin J-C, Toson B, Monnet E, et al. Farmer's lung precipitins in Doubs (a department of France): prevalence and diagnostic value. *Allergy* 1994;**49**:744–750.
- Marcen G, Simioni L, Saia B, Saladino G, Gemignani C, Mastrangelo G. Study of immunological parameters in farmer's lung. *Clin Allergy* 1983;**13**:443–449.