

Relationships of immunoglobulins E and G sensitization to respiratory function in dairy farmers

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ABSTRACT: An impairment of respiratory function has been demonstrated in dairy farmers. The objective of this study was to evaluate the relationship of allergy to respiratory function in dairy farmers in a longitudinal study conducted in the Doubs (France).

A cohort of male dairy farmers constituted in 1990 was re-evaluated in 1995. Subjects completed a medical and occupational questionnaire, and a spirometry test in both 1990 and 1995, in 1995 they were also subjected to immunological tests. Relationships between immunological variables and respiratory function were studied by a multiple linear regression model adjusted for age, smoking status, respiratory symptoms, altitude and occupational exposure. Amongst the 394 subjects of the initial cohort, 330 were included in the longitudinal study and 320 had immunological tests.

Log immunoglobulin (Ig) E was negatively correlated with the 1995 respiratory function parameters ($p < 0.05$ for forced expiratory volume in one second (FEV₁) and FEV₁/vital capacity (VC). Immunoglobulin (Ig) G response to *Aspergillus fumigatus* detected by enzyme-linked immunosorbent assay (ELISA) was negatively correlated to 1995 respiratory function parameters (VC: $p < 0.01$; FEV₁: $p < 0.001$; FEV₁/VC: $p < 0.01$). There was a positive relationship between IgG antibodies against *Aspergillus fumigatus* and the mean annual decline in FEV₁ ($p < 0.01$) and FEV₁/VC ($p < 0.01$).

To conclude, allergy may play a role in the impairment of respiratory function in dairy farmers of the Doubs and sensitization to *Aspergillus fumigatus* seems to constitute an independent risk factor for the development of airflow obstruction in this occupational setting.

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Many epidemiological studies recently reviewed [1] have shown that farming was consistently associated with an excess of respiratory symptoms, especially chronic bronchitis. However, influence of farming on respiratory function parameters still needs to be better defined. In controlled cross-sectional studies, expiratory flow rates are usually slightly lower in farmers than in nonfarming controls but there are few longitudinal studies. Nevertheless, an accelerated decline in expiratory flow rates and forced vital capacity (FVC) has been demonstrated in grain [2] and swine workers [3].

For dairy farming in the Doubs, two cross-sectional studies were conducted in two different geographical areas [4, 5]. They showed a significantly higher prevalence of chronic bronchitis and, to a lesser degree, of bronchial obstruction in dairy farmers. The longitudinal analysis of one of these two cohorts suggested that long-term occupational exposure significantly accelerates the decline in vital capacity (VC) and forced expiratory volume in male dairy farmers [6]. Aetiologic factors of respiratory function impairment in dairy farmers are unclear and the respective responsibility of host and occupational factors needs to be delineated with more precision. Farmers are

exposed to a wide variety of micro-organisms which can induce IgE or IgG mediated reactions. Atopy is considered to be a possible risk factor for the development of chronic obstructive pulmonary disease (COPD) or for an accelerated decline in lung function [7–9] but the role of IgG sensitization has never been investigated in the general population or in farmers.

Therefore, this longitudinal study was conducted to analyse the relationships between biological markers of immunoglobulins E and G sensitization and respiratory function in dairy farmers.

Methods

This study was conducted in cooperation with the Mutualité Sociale Agricole (MSA) du Doubs, the French national insurance health mutual for farmers, whose medical department organizes annual free check-ups for all their members. The protocol was approved by the "Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale", the local review board for research involving human subjects. Informed written consent was obtained from each subject.

Study population

Population selection has been detailed in previous papers [10, 11]. A cohort of farmers was established in 1990, during MSA check-up sessions in five districts of the Doubs region. Subjects included were male farmers, working exclusively on dairy farms and involved in daily cattle foddering.

In 1995, each subject was contacted individually and invited to participate in an investigation identical to the one performed in 1990 (medical and occupational questionnaire, spirometric tests) [10], plus a blood test for immunological analyses. Subjects refusing to participate were contacted by phone in order to obtain demographic information and the reasons for their refusal. All examinations were performed on a morning January–May 1995.

Questionnaires

Questions on respiratory symptoms and definitions of chronic bronchitis, dyspnoea, asthma and semi-delayed respiratory symptoms have been previously described [11]. Smoking status was both analysed as a continuous variable in pack-yrs and as a 3-category variable. Non-smokers (NS) were defined as those having smoked on average less than one cigarette, one cigar, or one pipe a day for a year. Current smokers (CS) smoked this amount or more, and exsmokers (ES) had stopped smoking at least one month before the time at which they filled out the questionnaire. Alcohol consumption was analysed as a 3-category variable: <10 g, 10–100 g, and >100 g per day of the equivalent of pure alcohol.

The main questions of the occupational questionnaire concerned the size of the farm, the size of the herd, the method of storing and drying fodder (barn fodder drying or traditional drying), the type of tasks regularly performed (milking, foddering *etc.*). Geographical distribution was dichotomized according to altitude (plain or tableland). Exposure to fodder was estimated by the number of bale-years. The number of bale-years was the number of average density bales of hay effectively fed by the subject to the cattle per day, multiplied by the number of years of foddering. When farmers used round bales or loose hay, the equivalent number of average density bales was used. Average density bales weighed 15 kg and round bales 250 kg.

Respiratory function tests

Slow vital capacity (VC) and forced expiratory volume in one second (FEV₁) were measured according to the American Thoracic Society recommendations [12] using the same portable pneumotachograph (Autospiro Minato AS 500; Medical Science Company Ltd, Osaka, Japan) as that used in 1990. For cross-sectional comparisons, values were expressed as a percentage of the European Coal and Steel Community reference values, calculated in relation to gender, age and height [13]. The annual decline of respiratory function parameters was expressed as mL·year⁻¹ and calculated using the following formula: 1995 value–1990 value/(number of months between 1990 and 1995 examinations /12).

Immunological analyses

Venous blood samples were collected from each patient for total serum IgE determination, Phadiatop®, (Cap-system; Pharmacia Diagnostic, AB, Uppsala, Sweden) and detection of antibodies against *Aspergillus fumigatus* and *Micropolyspora faeni* (or *Faeni rectivirgula*) by the enzyme-linked immunosorbent assay (ELISA) and against two hay extracts from the Doubs by immunoprecipitating methods.

IgE mediated allergy. Total serum IgE levels were determined by a microparticle-enzyme-immuno-assay (MEIA Imx IgE Abbot, Rungis, France). According to the manufacturers recommendations, a total IgE concentration ≥ 180 kIU·L⁻¹ was considered as a sign of allergy. Analyses were successively performed using Log IgE as a continuous variable and total IgE as a quantitative variable with 180 kIU·L⁻¹ and 100 kIU·L⁻¹ as cut-off points. Specific IgE was detected by a combination of aeroallergens using the Phadiatop test (Phadiatop®), and results were expressed as positive or negative.

IgG mediated allergy. *Aspergillus fumigatus* (Longbottom strain) was cultivated on Panmede medium (Paynes and Byrne Ltd., Greenford, UK) and *Micropolyspora faeni* on agar solid medium [14]. Antigen extraction was performed in Coca fluid for 7 days (CINa 4 g·L⁻¹, CO₃HNa 0.7 g·L⁻¹, phenol 4 g·L⁻¹) and followed by repeated freezing and unfreezing during 6 days, filtration, dialysis and lyophilization [15]. Hay extracts were prepared by defatting by exposure to ether solution overnight, extraction in Coca fluid, filtration, dialysis and lyophilization [16].

Precipitins were classically detected by Ouchterlony's agar-gel double diffusion [17] and immunoelectrophoresis [18]. Plates were examined by two biologists of the Clermont-Ferrand immuno-allergology laboratory (Besançon, France) who counted the number of precipitation bands and analysed their intensity. The reaction was considered as being positive when there was at least one precipitation arc.

The ELISA method consisted of that described by RICHARDSON *et al.* [19] adapted for the antigens prepared in the present laboratory. Modification and validation of the method was performed using the chessboard titration method. Polystyrene microtitre plates, Imulon A for *Micropolyspora faeni* and Imulon II for *Aspergillus fumigatus* (Dynatech, Guyancourt, France), were incubated with the antigen at 10 µL·mL⁻¹ for 1 h 30 min at 37°C. Patient sera was diluted in PBS-Tween 20 at a concentration of 1/50 and each serum was incubated in 2 wells for 1 h 30 min at 37°C. Alkaline phosphatase conjugated goat anti-human IgG was then incubated for 1 h at 37°C and revealed by P-nitrophenyl-phosphate (Sigma, Saint-Quentin Fallavier, France). Between each step, plates were washed three times with phosphate buffered saline. Absorbency of each well was measured by an automatic microplate reader at 37°C (Kinetic QCL Bio-whittaker, Gagny, France). The optical density (OD) of each serum, was the mean of the OD measured in the 2 wells. A control antigen without serum and a highly positive control serum were placed into each plate. To

take into account the variance between assays, an index was calculated for each serum using the following formula [20]:

$$\text{Index} = \frac{\text{OD tested serum} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}} \times 100$$

Thresholds have been determined by previous assays using double-diffusion and immunoelectrophoresis as reference methods. The index value of 30% was used as the cut-off point for the two antigens.

Statistical methods

Farmers no longer exposed to fodder in 1995 were excluded from the analysis. Spirometric data considered as inadequate according to the recommendations used were also excluded [12].

Univariate and multivariate analyses were successively performed to evaluate the relationships of the immunological variables, individual and occupational characteristics and respiratory symptoms to respiratory function parameters in 1995 (VC, FEV₁, and FEV₁/VC) and their annual decline 1990–1995. Variables used for the cross-sectional study were those of 1995. As the IgE level did not have a normal distribution, the log IgE variable was used. For the longitudinal analysis, new variables were constructed. Smoking habits were categorized as follows: nonsmokers; exsmokers before 1990; exsmokers 1990–1995; current smokers having smoked <5 pack-yrs 1990–1995 and current smokers having smoked >5 pack-yrs between the two surveys. Exposure was estimated by the number of bale-years supplied during the five yrs. For symptoms, the following three categories were considered: absence of symptoms in 1995; emergence of symptoms 1990–1995; and presence of symptoms in 1990 and 1995. Univariate statistical methods included an unpaired t-test, analysis of variance, and simple linear regression.

Independent variables for which univariate analysis showed a significant relationship ($p < 0.05$) with respiratory function parameters were entered in a multiple linear regression. Adjustment was performed on variables significantly correlated with respiratory function in the univariate analysis and in the first study [11], and on those known to be strong determinants of respiratory function (age, altitude, smoking status and respiratory symptoms including chronic bronchitis, asthma and asthma symptoms).

Data analysis was performed using the BMDP statistical software package (BMDP Statistical softwareTM, BMDP, LA, USA). P-values ≤ 0.05 were considered to be significant.

Results

Characteristics of the study population

A total of 372 subjects (94%) of the original cohort were seen and re-examined at the second survey in 1995. The mean interval between the two examinations was 4.6 yrs \pm 0.3. The reasons for nonresponse to follow-up were

refusal (15 cases), death (5 cases), lost to follow-up (3 cases) and nonrespiratory severe illness (2 cases). Twenty-eight subjects were no longer exposed to fodder and were excluded from analysis; 24 subjects had effectively retired and 4 had changed jobs (2 due to semi-delayed respiratory symptoms). Fourteen farmers were excluded from spirometric parameter analysis. The reasons for exclusion were missing data in 1990 or in 1995 (9 cases), or measures considered as inadequate according to the adopted criteria (5 cases). Among the 330 subjects left, 10 did not have immunological analyses in 1995, either due to blood test refusal (2 cases) or failure or insufficient quantity of blood. Finally, relationships between decline in lung function and sensitization were analysed in 320 farmers.

Comparisons between the study group and the farmers excluded or lost was performed for the 1990 values. Re-evaluated subjects were younger and less frequent smokers. They lived at higher altitudes, more frequently used artificial drying methods, and had more cattle, but cumulative exposure to fodder was no different. The re-evaluated group was less symptomatic (significantly for chronic bronchitis, $p < 0.01$). All respiratory function parameters were significantly higher in the re-evaluated group compared with the nonre-evaluated subjects (mean VC (% pred) 103.1 *versus* 96.65, $p < 0.01$; mean FEV₁ (% pred) 97.2 *versus* 87.4, $p < 0.001$ and mean FEV₁/VC (% pred): 94.8 *versus* 76.8, $p = 0.05$, respectively).

The individual, occupational and medical characteristics of subjects included in the longitudinal analysis are detailed in table 1. By univariate analysis, 1995 respiratory function parameters were found to be significantly lower in subjects living at higher altitudes, in asthmatic farmers and in subjects who had symptoms at exposure (wheezing with shortness of breath or cough). Annual decline in respiratory function parameters was positively correlated with altitude (VC and FEV₁), alcohol consumption (FEV₁ and FEV₁/VC), age (FEV₁ and FEV₁/VC), symptoms at exposure (FEV₁/VC), chronic bronchitis, semi-delayed respiratory symptoms and smoking status (VC). All these parameters that significantly influenced the respiratory function parameters were included in the multivariate analysis. Variables shown to be significantly correlated with the respiratory function parameters in the multivariate analysis were: altitude (Δ VC and Δ FEV₁), alcohol consumption (Δ FEV₁/VC), asthma (FEV₁ (% pred) and FEV₁/VC (% pred)) and symptoms at exposure (Δ FEV₁/VC).

Relationships of immunoglobulins E and G sensitization to respiratory function: univariate analysis

Results of IgE and IgG measurements and the Phadiatop[®] test are presented in table 2. Correlations between all listed variables were systematically tested. No relationships were identified between IgE and IgG response indicators. Within IgE mediated allergy, Phadiatop[®] and total IgE level were positively and closely associated. Within IgG sensitization, *Aspergillus fumigatus* and *Micropolyspora faeni* IgG antibodies were positively correlated. Precipitins directed against hay extracts were not linked to any other immunological variable.

Relationships between immunological variables and the 1995 respiratory function parameters and their evolution

Table 1. – Individual and occupational characteristics and symptoms in 1995

Characteristics	
Subjects n	330
Age	48±12
Smoking status	
Nonsmokers	201 (60.9)
Exsmokers	55 (16.7)
Current smokers	74 (22.4)
Pack-years*	15±11.7
Alcohol	
<10 g·day ⁻¹	133 (40.3)
10–100 g·day ⁻¹	149 (45.2)
>100 g·day ⁻¹	48 (14.5)
Plain/Tableland	51 (15.5)/279 (84.5)
Occupational characteristics	
Cattle	76.6±40.8
Bale-years	1020±571
Traditional/barn fodder drying	219 (66.4)/111 (33.6)
Foddering: yes/no	322 (97.6)/8 (2.4)
Symptoms	
Dyspnoea	32 (9.9)
Chronic bronchitis	23 (7.2)
Asthma	13 (3.9)
Semi-delayed symptoms†	12 (3.6)
Immediate symptoms‡	92 (27.9)
Rhinitis or conjunctivitis	157 (47.9)

Data are presented as mean±SD. Data in parentheses are presented as n(%). SD: Standard deviation. *: In smokers and exsmokers; †: cough and dyspnoea 4–10 h after exposure; ‡: cough or shortness of breath with wheeze during exposure.

1990–1995 are detailed in tables 3 and 4. Respiratory function parameters in 1995 were negatively correlated with IgE mediated allergy and IgG antibodies against *Aspergillus fumigatus*. No relationships were found between precipitins directed against hay extracts or *Micropolyspora faeni* antibodies and respiratory function parameters. There was a highly significant positive correlation between IgG antibodies against *Aspergillus fumigatus* and the annual decline in FEV₁ and FEV₁/VC. In the subgroup of nonsmokers, there was an accelerated decline in expiratory flow rates in the case of *Aspergillus fumigatus* positive serology (mean Δ FEV₁±SD: -62.53±99.08 versus -26.62±69.74 mL·yr⁻¹, p=0.04; mean Δ FEV₁/VC±SD: -1.46±2.03 versus -0.40±1.70 mL·yr⁻¹, p=0.001). When *Micropolyspora faeni* antibodies were positive in nonsmokers, there was a trend for an accelerated decline in FEV₁ when compared with nonsmoking nonsensitized subjects (mean±SD: -24.15±94.81 versus -2.20±94.77 mL·yr⁻¹, p=0.15 for VC; -49.16±80.26 versus -27.82±76.52 mL·yr⁻¹, p=0.09 for FEV₁; and -0.74±1.98 versus -0.57±1.75 mL·yr⁻¹, p=0.56 for FEV₁/VC, respectively).

Relationships of immunoglobulins E and G sensitization to respiratory function: multivariate analysis

As the Phadiatop® and IgE levels were closely linked, they were tested separately in the model. Log IgE was negatively correlated with FEV₁ and FEV₁/VC but there was no significant relationship between Log IgE and annual decline in respiratory function (table 5). Identical results were observed with the Phadiatop® test.

Table 2. – Immunoglobulins E and G sensitization

Sensitization variables	
IgE mediated allergy n	315
Total IgE ≥180 kIU·L ⁻¹	50 (15.8)
Total IgE ≥100 kIU·L	93 (29.5)
Total IgE mean (95% CI)	147 (102.24–192.81)
Positive Phadiatop	73 (23.2)
IgG mediated allergy n	320
<i>Aspergillus fumigatus</i> IgG antibodies	
ELISA	67 (20.9)
ELISA index mean	20.5 (18.19–22.77)
<i>Micropolyspora faeni</i> IgG antibodies	
ELISA n (%)	78 (24.4)
ELISA index mean (95% CI)	22.9 (19.42–26.41)
Plain hay extracts IgG antibodies	
Precipitins	155 (48.4)
Tableland hay extracts IgG antibodies	
Precipitins	197 (61.6)

Data in parentheses are presented as n(%). IgE: immunoglobulin E; IgG: immunoglobulin G; ELISA: enzyme-linked immunosorbent assay; CI: confidence interval.

IgG antibodies to *Aspergillus fumigatus* were shown to be negatively correlated with 1995 respiratory function values and positively with annual decline in FEV₁ and FEV₁/VC. No relationships were observed between IgG sensitization against *Micropolyspora faeni* and respiratory function parameters, regardless of the method used.

Discussion

This longitudinal study shows that male dairy farmers from the Doubs who have IgG antibodies against *Aspergillus fumigatus* have significantly lower respiratory volumes, decreased expiratory flow rates and an accelerated decline in expiratory flow rates. These results suggest that IgG mediated allergy to *Aspergillus fumigatus* may be a risk factor for the development of airflow obstruction, independent of environmental and occupational factors, smoking status and respiratory symptoms.

Although smoking is the main cause of chronic airflow obstruction, only a minority of smokers develop a chronic obstructive pulmonary disease (COPD) [21]. This means that host factors play an important role. Results of cross-sectional and longitudinal studies conducted in the general population and in the working population suggest that nonspecific bronchial hyperresponsiveness and atopy may be independent risk factors for COPD [7]. High IgE levels could indicate the presence of a disease process that may involve bronchial inflammation, which impairs lung function over time [22]. Few studies have been performed in farmers. In Finnish dairy farmers, one cross-sectional study [23] and one longitudinal study [24] have shown that atopy (defined clinically and/or by positive skin prick tests to usual aeroallergens) was significantly correlated with chronic bronchitis. In an analysis by the same Finnish group, atopy was identified as a potential risk factor for distal airflow obstruction, but no significant relationship was found between atopy and FEV₁ impairment [25].

In this study, total IgE level was negatively correlated with the respiratory function parameters in 1995. This is

Table 3. – Influence of immunoglobulins E and G sensitization on respiratory function: univariate analysis

	%VC*	p-value	%FEV1*	p-value	%FEV1/VC*	p-value
IgE ≥ 100 KUI-L	99.7 \pm 10	0.2	95.7 \pm 12	0.07	96.0 \pm 9	0.26
IgE <100 KUI-L	101.8 \pm 13		98.9 \pm 15		97.4 \pm 10	
IgE ≥ 180 KUI-L	98.2 \pm 9	0.05	93.4 \pm 10	0.03	93.4 \pm 9	0.20
IgE <180 KUI-L	101.3 \pm 13		98.3 \pm 15		97.3 \pm 10	
Log IgE	0.07 \pm 12	0.3	-0.15 \pm 14	0.009	-0.15 \pm 10	0.008
Phadiatop ⁺	97.6 \pm 11	0.2	94.4 \pm 12	0.03	96.2 \pm 8	0.4
Phadiatop ⁻	101.6 \pm 12		98.5 \pm 14		97.3 \pm 10	
ELISA AF ⁺	97.6 \pm 13	0.01	92.2 \pm 18	0.005	93.8 \pm 12	0.01
ELISA AF ⁻	101.8 \pm 12		99.1 \pm 12		97.8 \pm 9	
ELISA index AF	-0.18 \pm 12	0.001	-0.24 \pm 14	<0.001	-0.18 \pm 10	0.001
ELISA MF ⁺	98.7 \pm 12	0.07	96.9 \pm 14	0.6	98.2 \pm 11	0.2
ELISA MF ⁻	101.6 \pm 12		97.9 \pm 14		96.5 \pm 9	
ELISA index MF	-0.11 \pm 12	0.05	-0.09 \pm 14	0.12	-0.01 \pm 10	0.8
Precipitins EF ₁ ⁺	100.7 \pm 13	0.8	97.6 \pm 15	0.9	96.9 \pm 10	1
Precipitins EF ₁ ⁻	101.1 \pm 12		97.7 \pm 13		96.9 \pm 9	
Precipitins EF ₂ ⁺	100.6 \pm 13	0.5	97.6 \pm 14	0.9	97.3 \pm 9	0.43
Precipitins EF ₂ ⁻	101.4 \pm 11		97.8 \pm 14		97.4 \pm 10	

* 1995 respiratory function parameters are expressed as percentages \pm SD of the European Community for Coal and Steel reference values [13]. For continuous variables (log IgE, ELISA index AF, ELISA index MF), values are regression coefficients \pm SEM. AF: *Aspergillus fumigatus*; MF: *Micropolyspora faeni*; EF₁: plain hay extracts; EF₂: tableland hay extract; IgE: immunoglobulin E; VC: vital capacity; FEV₁: forced expiratory volume in one second; +: positive reaction; -: negative reaction; ELISA: enzyme-linked immunosorbent assay.

consistent with the results of the studies cited previously. However, total IgE determination was shown not to have a statistically significant relationship with the decline in respiratory function parameters. The most original finding of this study is the correlation of IgG mediated allergy with ventilatory impairment. IgG antibodies against *Aspergillus fumigatus* seem to be an independent risk factor for a decline in the respiratory function parameters, especially expiratory flow rates. One limitation of the study is that immunological tests were performed at the end of the study period. Theoretically, the decline in respiratory function parameters and development of chronic airflow obstruction might be the cause of IgG mediated allergy and not the consequence hence, this has to be considered. However,

such an hypothesis has no scientific logic. Subjects with an accelerated decline in respiratory function might reduce their occupational exposure and would therefore, be less likely to develop antibodies. This reduction in exposure can only have undermined the observed relationship.

Another potential bias is that subjects who were not seen at the second evaluation had a worse respiratory function than re-evaluated subjects. This selection of healthier subjects may have induced a loss of power for the analysis and may have led to an underestimation of the results. Smoking may represent another cause of underestimation of the results as stronger relationships between IgG sensitization and annual decline in respiratory function were found in the subgroup of nonsmokers, especially for

Table 4. – Influence of immunoglobulins E and G sensitization on annual decline in respiratory function 1990–1995: univariate analysis

	Δ VC*	p-value	Δ FEV1*	p-value	Δ FEV1/VC*	p-value
IgE ≥ 100 KUI-L	-17.1 \pm 97	0.61	-35.6 \pm 76	0.94	-0.58 \pm 2	0.14
IgE <100 KUI-L	-7.0 \pm 109		-36.4 \pm 76		-0.70 \pm 2	
IgE ≥ 180 KUI-L	-17.9 \pm 113	0.87	-49 \pm 68	0.23	-0.75 \pm 2	0.59
IgE <180 KUI-L	-15.4 \pm 96		-34.8 \pm 78		-0.59 \pm 2	
Log IgE	0.006 \pm 90	0.9	0.05 \pm 16	0.4	0.04 \pm 2	0.5
Phadiatop ⁺	-22.6 \pm 106	0.5	-40.5 \pm 72	0.6	-0.6 \pm 2	1
Phadiatop ⁻	-14 \pm 97		-35.4 \pm 77		-0.6 \pm 2.9	
ELISA AF ⁺	-3.53 \pm 92	0.3	-57.5 \pm 85	0.01	-1.3 \pm 2	0.001
ELISA AF ⁻	-17.2 \pm 100		-30.9 \pm 73		-0.4 \pm 2	
ELISA index AF	0.011 \pm 101	0.8	-0.18 \pm 77	0.001	-0.16 \pm 2	0.006
ELISA MF ⁺	-16.6 \pm 92	0.8	-42.6 \pm 77	0.4	-0.67 \pm 2	0.8
ELISA MF ⁻	-13.5 \pm 103		-34.5 \pm 76		-0.62 \pm 2	
ELISA index MF	-0.03 \pm 100	0.6	-0.07 \pm 77	0.2	-0.03 \pm 2	0.6
Precipitins EF ₁ ⁺	-9.7 \pm 105	0.4	-34.2 \pm 78	0.48	-0.68 \pm 2	0.7
Precipitins EF ₁ ⁻	-18.6 \pm 95		-38.8 \pm 75		-0.58 \pm 2	
Precipitins ₂ ⁺	-12.8 \pm 101	0.7	-35.2 \pm 77	0.7	-0.6 \pm 2	0.9
Precipitins EF ₂ ⁻	-16.7 \pm 98		-38.7 \pm 76		-0.6 \pm 2	

*: Mean annual decline in respiratory function in mL \cdot yr⁻¹ \pm SD for VC and FEV₁ and in % \cdot yr⁻¹ \pm SD for FEV₁/VC. For continuous variables (log IgE, ELISA index AF, ELISA index MF), values are regression coefficients \pm SEM. AF: *Aspergillus fumigatus*; MF: *Micropolyspora faeni*; EF₁: plain hay extracts; EF₂: tableland hay extracts; IgE: immunoglobulin E; ELISA: enzyme-linked immunosorbent assay; VC: vital capacity; FEV₁: forced expiratory volume in one second; +: positive reaction; -: negative reaction.

Table 5. – Influence of sensitization variables on respiratory function parameters: multivariate analysis*

	VC		FEV ₁		FEV ₁ /VC	
	%VC ⁺	ΔVC [‡]	%FEV ₁ [†]	ΔFEV ₁ [‡]	%FEV ₁ /VC [†]	ΔFEV ₁ /VC [‡]
Log IgE						
RC	-0.32	-1.40	-1.1	-0.50	-0.8	-0.002
SE	0.5	3.9	0.55	3	0.38	0.09
p-value	0.5	0.73	<0.05	0.87	<0.05	0.98
ELISA Index AF						
RC	-0.1	0.19	-0.16	-0.60	-0.08	-0.015
SE	0.3	3.9	0.04	0.20	0.03	0.005
p-value	<0.01	0.73	<10 ⁻⁴	<0.01	<0.01	<0.01
ELISA Index MF						
RC	-0.004	-0.13	-0.03	-0.06	-0.002	0.0009
SE	0.02	0.18	0.02	0.13	0.02	0.003
p-value	0.12	0.94	0.23	0.67	0.93	0.79
Constant	100.3	49.54	106.28	43.63	106.97	0.72
r ²	7%	11%	15%	13%	11%	14%

*Multiple linear regression: all listed immunological variables were simultaneously included in the model. All regression coefficients (RC), standard errors (SE) and p-values were adjusted for the other listed immunological variables, age, altitude, smoking status, alcohol, size of the herd and respiratory symptoms (chronic bronchitis, asthma and symptoms at exposure). [†]: 1995 values expressed as a percentage of the European Community for Coal and Steel reference value [13]; [‡]: mean annual decline in mL·yr. AF: *Aspergillus fumigatus*; MF: *Micropolyspora faeni*.

Micropolyspora faeni. Smoking has to be considered as a confounding variable. It is well known that an accelerated decline is observed in smokers and that nonsmokers are more likely to develop antibodies to inhaled antigens [1].

Several hypotheses can be proposed to explain this highly significant relationship between sensitization against *Aspergillus fumigatus* and respiratory function. IgG antibodies directed against *Aspergillus fumigatus* may reflect subclinical alveolitis, which would induce a progressive decline in respiratory function parameters. The fact that a significant relationship between farmer's lung and COPD (chronic bronchitis and emphysema) has been demonstrated both in the Doubs [26] and in other regions [27–29] could argue in favour of this hypothesis. The development of antibodies directed against *Aspergillus fumigatus* may only reflect an individual predisposition towards developing chronic airflow impairment, independent of the occurrence of an allergic alveolitis. This second hypothesis would be consistent with the Dutch hypothesis for the development of COPD [30]. Finally, IgG sensitization could only represent an indication of exposure which may explain the observed relationship. This is unlikely since no relationship between respiratory function and self-reported estimation of exposure to fodder or cattle was observed.

A significant relationship between respiratory function and IgG mediated allergy was found only for *Aspergillus fumigatus*. In a previous study, *Micropolyspora faeni* has never been detected in the farms of the Doubs, in which 6 cases of farmer's lung were diagnosed [31]. Moreover, in another study conducted in the same region, the authors were unable to observe any relationship between precipitins to *Micropolyspora faeni* and respiratory symptoms [16]. *Micropolyspora faeni* is probably not responsible for allergic diseases in the Doubs. Sensitization against *Micropolyspora faeni* may therefore, be due to cross-reactivity with other bacteria [32, 33].

To conclude, the present results confirm the important role of allergy in the development of respiratory function

impairment in dairy farmers and suggest that subjects sensitized against *Aspergillus fumigatus* may be at risk of developing chronic obstructive pulmonary disease. This cohort of farmers will soon be re-evaluated to confirm these results. As *Aspergillus fumigatus* is a ubiquitous antigen, it would also be interesting to analyse the influence of sensitization against *Aspergillus fumigatus* on respiratory function in the general population.

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