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Abstract

Background: Although farming is often considered a risk factor for COPD, data regarding the burden and characteristics of COPD in dairy farmers are sparse and conflicting.

Objectives: To characterize COPD in dairy farmers.

Methods: 4788 subjects entered two parallel COPD screening programs, one in agricultural workers and one in general practice from 2011 to 2015. Subjects with COPD were invited to participate in the characterization phase of the study. Those who accepted were included in two subgroups: dairy farmers with COPD (DF-COPD) (n = 101) and non-farmers with COPD (NF-COPD) (n = 85). Patients with COPD were frequency-matched with subjects with normal spirometry for age, sex and tobacco smoking (pack-years and status) (DF-controls n = 98, NF-controls n = 89). All subjects from these four groups underwent lung function and exercise testing, questionnaires and blood analysis.

Results: The frequency of COPD in dairy farmers was 8.0% using the GOLD criterion and 6.2% using the lower limit of normal criterion and was similar in non-farming subjects (7.3% and 5.2%, respectively) although dairy farmers had lower tobacco consumption (screening phase). DF-COPD had better pulmonary function, exercise capacity and quality of life, fewer symptoms and comorbidities than NF-COPD, and higher levels of some Th2 biomarkers (MCP-2, periostin) (characterization phase). In farmers, COPD was not related to occupational exposure factors, supporting the role of host factors.

Conclusion: COPD secondary to organic dust exposure (dairy farming) appears less severe and associated with fewer comorbidities than COPD secondary to tobacco smoking.

Key words:
Chronic obstructive pulmonary disease
Environmental risk factors
Farming
Occupational risk factors
Organic dust

1. Introduction

The burden of chronic obstructive pulmonary disease (COPD) is very high in terms of morbidity, mortality and direct and indirect costs (Rabe and Watz, 2017; GBD 2017 DALYs and HALE Collaborators, 2018). Early diagnosis and appropriate management are encouraged to reduce mortality and disability and to improve patients' quality of life as well as their social and occupational life (Singh et al., 2019). A case-finding approach with spirometry has its place in identifying COPD in order to make an appropriate diagnosis in subjects who report exposure to at least one risk factor for the disease (Guillien et al., 2017).

Although tobacco smoking is the single most important causal factor for COPD, about 20% of cases can be attributed to non-smoking risk factors such as indoor and outdoor air pollutants and occupational causes such as dairy farming (Salvi and Barnes, 2009; Lamprecht et al., 2011). Several studies have indicated that the prevalence of persistent airflow limitation and of COPD is higher among dairy farmers than in the general population (Stoleski et al., 2015; Guillien et al., 2016; Marescaux et al., 2016; Guillien et al., 2019), although the prevalence of tobacco smoking is lower (Roux et al., 2017). As dairy farmers are exposed to high concentrations of organic dusts, this increased risk seems mainly driven by chronic exposure to these dusts (Basinas et al., 2014; Pfister et al., 2018). Organic dusts contain particles of plant, animal and microbial origin (Douwes et al., 2003). The main agent in organic dust is endotoxin, a major component of the outer membrane of Gram-negative bacteria, but components of Gram-positive bacteria (peptidoglycans) and fungi (glucans) are also present. An "immunoallergic" and inflammatory reaction following the inhalation of organic dust contained in hay has been proposed to explain this phenomenon (Dalphin et al., 1993; Eduard et al., 2009; Poole and Romberger, 2012). Bronchial hyperresponsiveness during exposure to organic particles could be an indicator associated with the subsequent onset of COPD (Dalphin et al., 1993; Westeel et al., 2000). However, current knowledge on occupational COPD in dairy farming remains sparse, not only with regard to its mechanisms but also to its specific clinical characteristics. We therefore conducted a prospective cross-sectional study (i) to assess the frequency of COPD among dairy farmers and (ii) to characterize COPD among dairy farmers in terms of dyspnea, quality of life, pulmonary function, exercise capacity and systemic inflammation, and to compare it with COPD secondary to tobacco.

2. Methods

2.1. Identification of patients and controls

The BALISTIC project (COPD in dairy farmers: screening, characterization and constitution of a cohort; ClinicalTrials.gov Identifier: NCT02540408) was conducted from 2011 to 2015 at the University Hospital of Besançon, France, in collaboration with the French national social security system for agricultural workers (Mutualité Sociale Agricole, MSA) and the federation of community health practices of Franche-Comté (Fédération des Maisons de Santé Comtoises, FeMaSaC) (Degano et al., 2012; Soumagne et al., 2019).

Subjects with COPD and matched controls were recruited through two parallel screening programs (the screening phase of the BALISTIC study) as described elsewhere (Soumagne et al., 2019). Inclusion criteria in the screening programs were: men or women aged 40-74 years, with no previous diagnosis of asthma or hypersensitivity pneumonitis, and who were either dairy farmers (dairy farmer subgroups) or unexposed to any of the occupational hazards that have ever been associated with COPD (non-farmer subgroups). One of these two screening programs was conducted during free health check-ups organized by the MSA for their affiliated members, where COPD screening with spirometry was proposed to all subjects who attended the health check-up and fulfilled the inclusion criteria. In parallel, general practitioners of the FeMaSaC conducted a second COPD screening program targeting all their patients who met the same inclusion criteria. For COPD screening, both programs used the same material, that is, a pneumotachograph (MedGraphics, MSE Medical, Strasbourg, France) calibrated at least once daily using a 3-L syringe, and the same protocol (Soumagne et al., 2019).

2.2. Study groups

A diagnosis of COPD was retained on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criterion or on the lower limit of normal (LLN) criterion (Rabe et al., 2007; Swanney et al., 2008). The GOLD criterion defines airflow limitation as a post-bronchodilator (post-BD) FEV_1/FVC ratio below the fixed value of 0.70. This fixed GOLD criterion does not consider the normal decline of FEV_1/FVC ratio with age. Thus, a lower limit of normal (LLN) for FEV_1/FVC that takes age into account is increasingly used to define airflow limitation (Quanjer et al., 2012). The LLN approach also allows calculation of a z-score, and airflow limitation is defined as FEV_1/FVC < LLN (i.e., z-score < -1.64). In practice, the use of the fixed ratio of 0.70 (GOLD) may result in more frequent diagnosis of airflow limitation in the elderly and less frequent diagnosis in younger adults. By contrast, using an LLN cut-off based on age results in more frequent diagnosis of airflow limitation in younger adults and less frequent diagnosis in older subjects (Mannino et al., 2007; Guillien et al., 2018). We therefore used both GOLD and LLN criteria to present the frequency of COPD.

Spirometry was considered normal when the FEV_1/FVC ratio was > 0.70 and FEV_1 was > 80% of the predicted value before bronchodilator administration according to the GLI-2012 equations (Quanjer et al., 2012; Singh et al., 2019).

During the study period, all subjects with COPD detected during the screening phase of the BALISTIC study were invited to participate in the characterization phase of the study. Those who accepted were included in two subgroups, namely dairy farmers with COPD (DF-COPD) and non-farmers with COPD (NF-COPD). Patients from these two groups were frequency-matched with subjects with normal spirometry (as defined above) in terms of age, tobacco smoking (in pack-years and smoking status) and sex; these two groups were named DF-controls and NF-controls. Patients were in stable condition (no exacerbation of COPD during the previous 6 weeks) and were not taking any oral or inhaled anti-inflammatory drugs, including oral corticosteroids. Subjects were asked to interrupt short and long-acting bronchodilators 72 hours prior to the visit. Exclusion criteria were long-term oxygen therapy, a history of asthma, pulmonary fibrosis, tuberculosis or bronchiectasis, and pregnancy in women.

Subjects of these 4 groups participated in the characterization phase of the study. They attended a visit at the University Hospital of Besançon, during which examinations and questionnaires were performed.

Ethical approval was received from the local Ethics Committee (CPP Est; 11/617), and written consent was obtained from all subjects.

2.3. Procedures

2.3.1. Lung function and exercise testing. Routine spirometry, constant-volume body plethysmography and single breath lung transfer for carbon monoxide (TLCO) were performed in accordance with current standards (Platinum Elite, MGC Diagnostics Corporation, Saint Paul, Minnesota, USA). Subjects with post-BD airway limitation were rated either as stage 1 (FEV_1 > 80% of the predicted value) or stage 2+ ($FEV_1 \leq 80\%$ of the predicted value). Symptom-limited incremental cardiopulmonary exercise testing (CPET) was performed on an electronically-braked cycle ergometer (Ergometrics 900, Ergoline, Bitz, Germany) as previously described (Soumagne et al., 2016).

2.3.2. Laboratory analysis. For each patient, blood gases were measured from samples drawn from the arterialized earlobe and measurements were corrected in order to estimate arterial oxygen partial pressure (PaO_2) (Aguilaniu et al., 2011). In addition, a venous blood sample was collected in the fasting state and serum samples were stored at -80°C for further analyses. The biomarkers periostin, monocyte chemoattractant protein 2 (MCP-2) and IgE were measured and were chosen as markers of Th2-type inflammation. Soluble tumor necrosis factor receptor 1 (sTNFR1), interleukin (IL)-6 and IL-8 were selected because they have been widely studied in COPD. Surfactant protein (SP-D), produced in type II pneumocytes, was selected as a marker of lung injury. Other common inflammatory biomarkers such as high-sensitivity C-reactive protein (hsCRP) and fibrinogen were also measured, because they are acute-phase reactants known to be associated with COPD. Periostin was measured using an ELISA kit (Thermo Scientific, Courtaboeuf, France) and IL-6, IL-8, sTNFR1, SP-D and MCP-2 were measured using a bead-based cytometric immunoassay (R&D Systems Europe, Abingdon, UK). Serum high-sensitivity C-reactive protein (hsCRP) concentrations were measured using a highly sensitive immunonephelometric assay.

2.3.3. Questionnaires. A medical questionnaire (completed by the physician) collected data on demographic characteristics, medical history, treatments, symptoms and smoking history. In addition, an adapted French translation of the long version of the European Community Respiratory Health Survey questionnaire was completed by the subjects (Dalphin et al., 1998). An occupational questionnaire was sent to the subjects 10 days before their medical examination, collected during the visit, and reviewed in their presence (Marescaux et al., 2016; Mauny et al., 1997).

2.3.4. Statistical analysis Data are presented as number (percent) and mean ± standard deviation for qualitative and quantitative variables, respectively. Quantitative variables were compared with the Student t test. Qualitative variables were compared with the Chi-square (χ^2) test. To identify factors associated with pulmonary function, exercise capacity and symptoms, linear regression analysis and logistic regression analysis for all candidate factors were performed. In addition, in order to take into account the confounding effect of tobacco smoking, a subgroup analysis between NF-COPD and non-smoking DF-COPD was performed.

A p value < 0.05 was considered statistically significant. Analyses were performed using SAS software (version 9.3, SAS Institute, Inc., Cary, NC, USA).

3. Results

Among the 8106 subjects who attended the screening sessions, 4788 met the inclusion criteria for the study and had interpretable spirometry (59.1%), including 3627 subjects recruited through the MSA program and 1161 subjects through the FeMaSaC program (Fig. 1). A diagnosis of COPD was found in 8.0% (GOLD definition) and in 6.2% (LLN definition) of dairy farmers and in 7.3% (GOLD) and 5.2% (LLN) of non-farmers (Fig. 1). All subjects who had COPD according to the LLN definition also had COPD according to the GOLD definition. The frequency of COPD, whether defined by GOLD or by LLN, was similar in dairy farmers and non-farmers. Nevertheless, prevalence of tobacco smoking was much lower in DF-COPD (21% of current smokers and 34% of subjects who had smoked more than 15 pack-years) than in NF-COPD (52% of current smokers and 72% of subjects who had smoked more than 15 pack-years; $p < 0.0001$ between farmers and non-farmers) (supplementary table S1).

3.1. Population characteristics

A total of 186 subjects with COPD and 187 matched controls with normal spirometry were included in the characterization phase of the study. All groups had similar sex distribution, age and BMI (Table 1). Among these 373 subjects included in the characterization phase of the study, the number of former and current smokers was much lower among dairy farmers than among non-farmers. In farmers as well as in non-farmers, the distribution of non-smokers, former smokers and current smokers was similar in subjects with COPD and in controls, but cumulative smoking (in pack-years) was higher in subjects with COPD than in controls (Table 1). Among non-farmers, 61% worked in the tertiary sector (mainly financial and professional services), 23% in the secondary sector (mainly car and watch manufacturing) and 16% were unemployed (supplementary Fig. S1). About 40% of patients with COPD had mild disease and 13% were taking long-term bronchodilators at the time of screening (supplementary Table S2).

3.2. Occupational characteristics of dairy farmers

Several occupational characteristics among dairy farmers were assessed (supplementary tables S3, S4 and S5). Activity level, farm characteristics, hay conditioning, exposure to organic dusts and chemical products were similar between DF-COPD and DF-controls.

3.3. Pulmonary function

A large majority of subjects with COPD had preserved FEV_1. Post-BD FEV_1 and TLCO were significantly higher in DF-COPD than in NF-COPD (Table 2). After adjustment on post-BD FEV_1, TLCO remained significantly higher in DF-COPD. Comparison between non-smoking DF-COPD and NF-COPD showed similar results, *i.e.*, a higher TLCO in non-smoking farmers with COPD than in non-farmers with COPD (supplementary Table S6).

3.4. Symptoms and quality of life

In DF-COPD, chronic bronchitis and wheezing were more frequent than in DF-controls but were similar to NF-COPD (Table 3). Frequency and severity of exacerbations were similar in

DF-COPD and NF-COPD. Dyspnea (assessed by BDI and mMRC) was less and quality of life (assessed by CAT and SGRQ) was less impaired in DF-COPD than in NF-COPD. Nevertheless, after adjustment on TLCO, post-BD FEV_1 or both, only BDI remained significantly better in DF-COPD ($p < 0.0001$).

3.5. Comorbidities

The main comorbidities in dairy farmers were hypertension, dyslipidemia and gastro-esophageal reflux; frequency of these comorbidities was similar in DF-COPD and DF-controls (Fig. 2). Diabetes mellitus and osteoporosis were less frequent in DF-COPD than in NF-COPD. In addition, DF-COPD were taking less medication (in particular anti-aggregates and proton pump inhibitors) than NF-COPD (supplementary Table S2).

3.6. Exercise capacity

All patients performed a maximal exercise test (respiratory exchange ratio > 1.1). Patients with COPD had significantly lower exercise capacity than controls (Table 4). DF-COPD had higher peak work rate (WR) and higher peak oxygen consumption (VO_2) than NF-COPD (Table 4). Nevertheless, after adjustment on both post bronchodilator FEV_1 and TLCO, only peak VO_2 remained higher in DF-COPD than in NF-COPD. Finally, the distance walked in 6 min was similar in both groups with COPD.

3.7. Systemic inflammation

Comparison of serum biomarkers showed that NF-COPD had higher levels of inflammatory markers (hsCRP and fibrinogen) than DF-COPD and controls (Fig. 3). By contrast, the levels of some Th2-related markers (periostin, MCP-2 and IgE) were higher in DF-COPD than in other subjects (Fig. 3).

4. Discussion

This study issued from large screening populations shows that (i) the frequency of COPD in dairy farmers was 8.0% using the GOLD criterion and 6.2% using the LLN criterion. It was similar to the prevalence observed in non-farmers, although tobacco consumption was markedly lower in farmers; (ii) DF-COPD had greater pulmonary function, exercise capacity and quality of life and fewer symptoms, comorbidities and systemic inflammation than non-farmers with COPD (mostly tobacco-related) matched on age, BMI and gender; (iii) COPD in dairy farmers is associated with some Th2 phenotype markers. In addition, (iv) the absence of relationship between COPD and occupational exposure factors in farmers supports the role of host factors in the pathogenesis of COPD in this specific population.

In the current study, we observed a prevalence of COPD among farmers that is in the range of previous studies (Eduard et al., 2009; Stoleski et al., 2015; Jouneau et al., 2019; Fix et al., 2020). Although farming is often considered a risk factor for COPD, there are few and conflicting data regarding COPD prevalence and the magnitude of the risk in dairy farmers. In a study performed in Macedonia, Stoleski et al. reported a COPD prevalence of 10.7% in dairy farmers whereas in a study performed in Brittany, Jouneau et al. found a COPD prevalence of only 1.3% (Stoleski et al., 2015; Jouneau et al., 2019). The differences in COPD prevalence could be due to differences in the level of organic dusts exposure in these different regions. In a previous study by our team, prevalence of COPD in farmers in fact varied from one region to another due to differences in farming activity (livestock, dairy production), meteorological data (climate, altitude), and farm size and modernity (Guillien et al., 2016; Marescaux et al., 2016). In our region, where farms are mainly traditional (Stein et al., 2016), prevalence of COPD among farmers is higher than in Brittany (Guillien et al., 2016). However, characteristics of the farm and level of organic dust exposure alone may not explain the occurrence of COPD in dairy farmers, as we found no difference in exposure between DF-COPD and controls. In addition, other data obtained in the same cohort as the current study showed similar exposure patterns between DF-COPD and DF-controls, and therefore exposure could not explain the occurrence of COPD (Barrera et al., 2018).

To our knowledge, this is the first study comparing clinical, functional and blood parameters among DF-COPD patients, tobacco-smoking COPD patients and healthy dairy farmers and non-farmers. Several studies have shown that dairy farming is associated with an excess of respiratory symptoms including chronic bronchitis and related symptoms (cough, phlegm, wheezing) compared with the general population (Dalphin et al., 1998; Chaudemanche et al., 2003; Guillien et al., 2019). In the current study we found a similar prevalence of respiratory symptoms between dairy farmers and non-farmers in the overall population.

Compared with NF-COPD, DF-COPD developed less severe airway limitation even though duration of exposure to toxic inhalants was similar in both groups. In addition, TLCO was significantly higher in DF-COPD even after adjustment on FEV_1. Low TLCO has been clearly linked to structural markers of early emphysema (Tan et al., 2015). We therefore suggest that dairy farming exposure, which has been shown to have pro-inflammatory effects *in vitro,* may be associated with a less damaging effect on lung parenchyma than tobacco smoking (Pfister et al., 2017). High resolution computed tomography (HRCT) was however not available for emphysema quantification, but is planned to be performed in the upcoming second part of the BALISTIC project (NCT03468101). Nevertheless, these results are in line with those found in COPD induced by exposure to biomass smoke, where emphysema is rarely found (Solleiro-Villavicencio et al., 2015; Olloquequi et al., 2018).

COPD is an inflammatory lung disease associated with systemic inflammation. It has been shown that levels of inflammatory markers such as TNFα, fibrinogen, IL-6 and CRP are higher than in non-COPD smokers, regardless of the severity of airway obstruction. The mechanisms of systemic inflammation are multifactorial and are still not completely understood. One hypothesis is that chronic inflammation arises from the spillover of inflammatory molecules from the lungs into the systemic circulation, while other mechanisms have been proposed including a direct effect of tobacco smoke on the activation of neutrophils or passage in the circulation of toxic gases or inhaled particles. In non-smoking COPD, it has been shown that patients exhibit higher levels of inflammatory biomarkers than healthy subjects (Montano et al., 2014; Golpe et al., 2017b). Systemic inflammation is somewhat lower in non-smoking than in smoking-induced COPD (Montano et al., 2014; Golpe et al., 2017b). In dairy farming, COPD was associated with a lower systemic inflammatory status than in NF-COPD. This could in part explain the lower prevalence of comorbidities in DF-COPD. A pathophysiological link between persistent low-grade systemic inflammation and comorbidities in COPD has in fact been suggested (Sin and Man, 2003; Gan et al., 2004). A similar lower prevalence of comorbidities was found in COPD induced by biomass exposure (Golpe et al., 2017a). It is plausible that the nature of the noxious agents (tobacco or organic dust exposure) could have a different impact on the occurrence of these comorbidities. In addition, it has been shown that people living and working on dairy farms have a rich and distinct nasal microbiome compared with that of non-farmers (Shukla et al., 2017). This increased microbial diversity may contribute to protection from chronic inflammatory conditions (Rook, 2010). Finally, we found that DF-COPD patients may have a predominantly Th2 phenotype, as has been shown in biomass COPD (Solleiro-Villavicencio et al., 2015). Periostin and MCP-2/CCL8, which are known to mediate Th2 inflammation with a specific potential to drive chronic eosinophilic inflammation, have been found at higher levels in DF-COPD (Debes and Diehl, 2011; de Llano et al., 2018). In addition, we found in the same population that IgE-mediated reactions (atopy) may play a role in the development of COPD in dairy farming (Veil-Picard et al., 2019).

All reasonable precautions were taken to verify the absence of asthma, including spirometry performed by trained nurses and careful medical examination. All subjects with self-reported asthma were excluded. Our findings therefore reinforce the so-called "Dutch hypothesis" that postulates that chronic obstructive airway disease may occur on a continuum, with "pure" forms of asthma and COPD representing the extremes of the same disease and the various forms of chronic obstructive airway disease being different expressions of a single disease entity (Orie et al., 1961; Postma et al., 2015).

Our study has limitations inherent to large screening programs. Only subjects who agreed to undergo a health check-up were included in the COPD screening program. In addition, only half the subjects who had COPD detected by screening agreed to attend the hospital visit for characterization. It has previously been reported that among all subjects who were invited to health check-ups organized by the French agricultural health insurance system, those who attend such check-ups and those who do not have different health characteristics (Guillien et al., 2016). Our study population, however, did not differ from the target population in terms of smoking habits.

5. Conclusion

This study demonstrates that dairy farmers have a similar frequency of COPD (8%) as unexposed subjects despite a much lower prevalence of tobacco smoking. Dairy farming therefore represents a relevant cause of occupational COPD. Patients with COPD secondary to organic dust exposure (*i.e.* dairy farming) have better lung function and quality of life and fewer comorbidities than patients with COPD secondary to tobacco smoking. This suggests that dairy farming may exert less damaging effects on lung parenchyma than tobacco smoking over a similar duration. Differences in biomarkers suggest that the inflammatory response induced by exposure to organic dust is more Th2-oriented than in tobacco smoking-induced COPD.

Author Contributions:

All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors made the following contributions:

JCD and BD made substantial contributions to the conception and design of the work.

TS and AG performed all analyses.

JCD and BD obtained study funding.

All authors made substantial contributions to the acquisition, analysis, or interpretation of data for the work. TS wrote the first draft of the manuscript. BD, TS, AG, IAM, PA, SH, OA, SJ, MB, JJL, NR and JCD revised the manuscript for important intellectual content. All authors approved the manuscript.

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Table 1
Main characteristics of the population.

	Dairy farmers COPD n = 101	Dairy farmers Controls n = 98	p	Non-farmers COPD n = 85	Non-farmers Controls n = 89	p	Dairy farmers vs non-farmers with COPD p
Demographics							
Male	86 (85)	86 (88)	NS	66 (78)	65 (73)	NS	NS
Age, years	60.3 ± 9.1	59.3 ± 8.9	NS	61.4 ± 7.6	60.2 ± 6.5	NS	NS
Body mass index, kg.m^{-2}	26.5 ± 4.1	27.0 ± 4.1	NS	26.1 ± 4.1	26.8 ± 3.8	NS	NS
Exposure							
Smoking status			NS			NS	**< 0.0001**
Non-smoker	48 (48)	54 (55)		7 (8)	12 (13)		
Former smoker	33 (33)	32 (33)		36 (42)	42 (47)		
Current smoker	20 (20)	12 (12)		42 (49)	35 (39)		
Tobacco, pack-years	12.5 ± 16.8	8.1 ± 12.1	**< 0.05**	31.8 ± 23.8	24.7 ± 17.7	**< 0.05**	**< 0.0001**
Exposure to organic dust, years	37.9 ± 6.1	36.7 ± 7.1	NS	-	-	-	-
Severity of airway limitation							NS
Stage I	44 (44)			33 (39)			
Stage II +	57 (56)			52 (61)			

Values are means ± SD or number (percent).
COPD, chronic obstructive pulmonary disease.

Table 2 Pulmonary function tests.

	Dairy farmers			Non-farmers			Dairy farmers vs non-farmers with COPD
	COPD n = 101	**Controls n = 98**	**p**	**COPD n = 85**	**Controls n = 89**	**p**	**p**
Post-BD FEV_1, L	2.84 ± 0.72	3.46 ± 0.69	< 0.0001	2.58 ± 0.76	3.42 ± 0.68	< 0.0001	**< 0.05**
z-score	-0.99 ± 0.98	0.38 ± 0.77	< 0.0001	-1.31 ± 1.05	0.58 ± 0.85	< 0.0001	**< 0.05**
Post-BD FEV_1/FVC, %	63 ± 7	79 ± 4	< 0.0001	61 ± 8	80 ± 4	< 0.0001	NS
z-score	-1.95 ± 0.78	0.07 ± 0.64	< 0.0001	-2.18 ± 0.86	0.27 ± 0.59	< 0.0001	NS
Pre-BD pulmonary function							
FEV_1, L	2.63 ± 0.72	3.37 ± 0.66	< 0.0001	2.41 ± 0.73	3.31 ± 0.66	< 0.0001	NS
z-score	-1.39 ± 1.03	0.18 ± 0.74	< 0.0001	-1.66 ± 1.04	0.34 ± 0.83	< 0.0001	NS
FVC, L	4.36 ± 1.11	4.48 ± 0.87	NS	4.08 ± 1.09	4.31 ± 0.9	NS	NS
z-score	0.12 ± 1.15	0.43 ± 0.77	< 0.05	-0.13 ± 1.11	0.42 ± 0.82	< 0.001	NS
FRC, L	4.07 ± 0.81	3.71 ± 0.78	< 0.01	4.13 ± 0.88	3.55 ± 0.83	< 0.001	NS
z-score	1.14 ± 1.24	0.54 ± 1.12	< 0.0001	1.32 ± 1.15	0.45 ± 1.18	< 0.001	NS
IC, L	3.31 ± 0.79	3.38 ± 0.7	NS	3.01 ± 0.83	3.34 ± 0.72	< 0.01	**< 0.05**
RV, L	2.87 ± 0.71	2.47 ± 0.58	< 0.01	2.89 ± 0.81	2.47 ± 0.57	< 0.01	NS
z-score	1.42 ± 1.78	0.47 ± 1.29	< 0.0001	1.48 ± 1.79	0.57 ± 1.85	< 0.001	NS
TLC, L	7.38 ± 1.27	7.10 ± 1.16	NS	7.14 ± 1.33	6.90 ± 1.25	NS	NS
z-score	1.13 ± 1.40	0.87 ± 1.05	NS	0.99 ± 1.28	0.89 ± 1.33	NS	NS
RV/TLC, %	39.2 ± 8.5	34.8 ± 6.1	< 0.0001	40.7 ± 8.8	35.8 ± 8.9	< 0.0001	NS
TLCO, mmol/min/kPa	8.45 ± 2.35	9.47 ± 2.12	< 0.01	6.74 ± 2.37	8.33 ± 2.39	< 0.0001	**< 0.0001**
z-score	-0.26 ± 1.33	0.56 ± 1.16	< 0.0001	-1.45 ± 1.40	-0.04 ± 1.42	< 0.0001	**< 0.0001**
KCO, mmol/min/kPa/L	1.34 ± 0.25	1.47 ± 0.23	< 0.001	1.13 ± 0.25	1.37 ± 0.26	< 0.0001	**< 0.0001**
z-score	-0.47 ± 1.13	0.08 ± 1.02	< 0.001	-1.44 ± 1.13	-0.35 ± 1.13	< 0.0001	**< 0.0001**
Arterial blood gases							
$PcapO_2$, kPa	81.0 ± 9.8	85.5 ± 9.8	< 0.01	79.5 ± 9.8	84.0 ± 9.8	< 0.01	NS
$PcapCO_2$, kPa	36.8 ± 3.8	36.8 ± 3.0	NS	37.5 ± 3.8	37.5 ± 3.8	NS	NS

COPD, chronic obstructive pulmonary disease; BD, bronchodilator; FEV_1, forced expiratory volume in 1 second; z-score, standardized residual; FVC, forced vital capacity; FRC, functional residual capacity; IC, inspiratory capacity; RV, residual volume; TLC, total lung capacity; TLCO, lung transfer for carbon monoxide; KCO, carbon monoxide transfer coefficient.
Values are means ± SD or number (percent).

Table 3 Symptoms and quality of life.

	Dairy farmers			Non-farmers			Dairy farmers vs non-farmers with COPD
	COPD n = 101	**Controls n = 98**	**p**	**COPD n = 85**	**Control n = 89**	**p**	**p**
Dyspnea	38 (38)	15 (15)	< 0.001	39 (46)	19 (21)	< 0.001	NS
mMRC	0.41 ± 0.55	0.15 ± 0.36	< 0.001	0.61 ± 0.77	0.25 ± 0.57	< 0.001	**< 0.05**
BDI	10.8 ± 1.4	11.5 ± 1.0	< 0.0001	10.1 ± 2.1	11.3 ± 1.4	< 0.0001	**< 0.01**
Wheezing							
Ever	32 (32)	6 (6)	< 0.0001	25 (29)	10 (11)	< 0.01	NS
In the last 12 months	30 (30)	11 (11)	< 0.01	28 (33)	15 (17)	< 0.01	NS
Chronic bronchitis, n (%)	35 (35)	9 (9)	< 0.0001	32 (38)	12 (13)	< 0.001	NS
Exacerbation, n (%)	23 (23)			23 (27)			NS
In the last 12 months	1.6 ± 1.2			1.4 ± 0.9			NS
Nasal sinus symptoms	27 (27)	17 (18)	NS	31 (36)	19 (21)	NS	NS
Hay fever	9 (9)	10 (10)	NS	12 (14)	11 (13)	NS	NS
Quality of life/prognosis							
SGRQ	15.1 ± 12.9	7.3 ± 7.2	< 0.0001	19.4 ± 14.9	9.3 ± 9.3	< 0.0001	**< 0.05**
CAT	9.8 ± 5.7			11.7 ± 5.7			**< 0.05**
BODE index	0.2 ± 0.6			0.4 ± 0.5			**< 0.05**

COPD, chronic obstructive pulmonary disease; mMRC, modified Medical Research Council scale; BDI, baseline dyspnea index; SGRQ, St. George's Respiratory Questionnaire; CAT, COPD Assessment Test; BODE index, body mass, airflow obstruction, dyspnea and exercise capacity.

Values are means ± SD or number (percent).

Table 4 Exercise capacity.

	Dairy farmers			Non-farmers			Dairy farmers vs non-farmers with COPD
	COPD n = 101	Controls n = 98	p	COPD n = 85	Controls n = 89	p	p
CPET (measurements at peak exercise)							
Work rate, W	159 ± 46	171 ± 46	NS	140 ± 47	161 ± 46	**< 0.01**	**< 0.01**
% predicted	85 ± 16	92 ± 17	**< 0.01**	77 ± 19	89 ± 19	**< 0.01**	**< 0.01**
VO_2, L/min	2.18 ± 0.57	2.36 ± 0.56	**< 0.05**	1.92 ± 0.56	2.23 ± 0.57	**< 0.001**	**< 0.01**
% predicted	99 ± 17	108 ± 19	**< 0.001**	90 ± 19	104 ± 20	**< 0.0001**	**< 0.01**
HR, % predicted maximum	97 ± 10	99 ± 7	NS	95 ± 10	99 ± 10	NS	NS
$PcapO_2$, kPa	11.8 ± 1.3	12.4 ± 1.1	**< 0.001**	11.5 ± 1.4	12.6 ± 1.3	**< 0.0001**	NS
$PcapCO_2$, kPa	4.8 ± 0.6	4.7 ± 0.5	NS	4.8 ± 0.6	4.7 ± 0.6	NS	NS
Reason for stopping*							
Leg discomfort	41 (56)	52 (68)	NS	27 (46)	49 (62)	NS	NS
Breathing discomfort	18 (25)	6 (8)	**< 0.01**	12 (21)	22 (28)	NS	NS
Both breathing and leg discomfort	5 (7)	7 (9)	NS	11 (19)	4 (5)	**< 0.05**	**< 0.01**
Other reason	9 (12)	11 (15)	NS	8 (14)	4 (5)	NS	NS
6MWT							
Distance, m	603 ± 85	617 ± 70	NS	604 ± 85	620 ± 85	NS	NS
% of predicted distance	88 ± 9	91 ± 10	NS	90 ± 10	93 ± 10	NS	NS
SpO_2 at rest, %	96 ± 2	96 ± 2	NS	96 ± 2	96 ± 2	NS	NS
Minimum SpO_2 during walk test, %	93 ± 3	94 ± 3	**< 0.01**	93 ± 3	94 ± 3	**< 0.01**	NS

COPD, chronic obstructive pulmonary disease; CPET, cardiopulmonary exercise testing; VO_2, oxygen uptake; HR, heart rate; $PcapO_2$, arterialized partial pressure of oxygen; $PcapCO_2$, arterialized partial pressure of carbon dioxide; 6MWT, 6 minutes walk test; SpO_2, oxygen saturation.

Figure legends

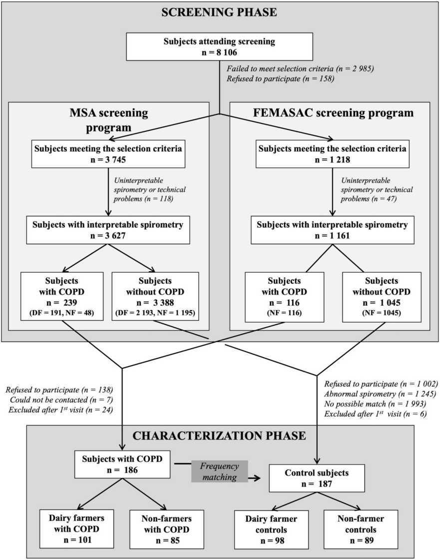
Fig. 1. Flow chart of participants included in the study. Subjects were recruited through screening programs in two social security organisms. Inclusion criteria in the screening programs were: men or women aged 40 to 74 years, with no history of chronic respiratory disease including asthma and hypersensitivity pneumonitis.

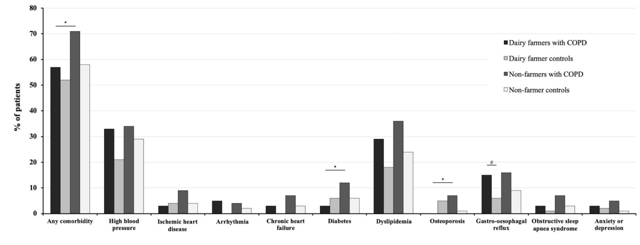
COPD, chronic obstructive pulmonary disease; DF, dairy farmer; NF, non-farmer.

Fig. 2. Prevalence of comorbidities in patients with COPD and control subjects.

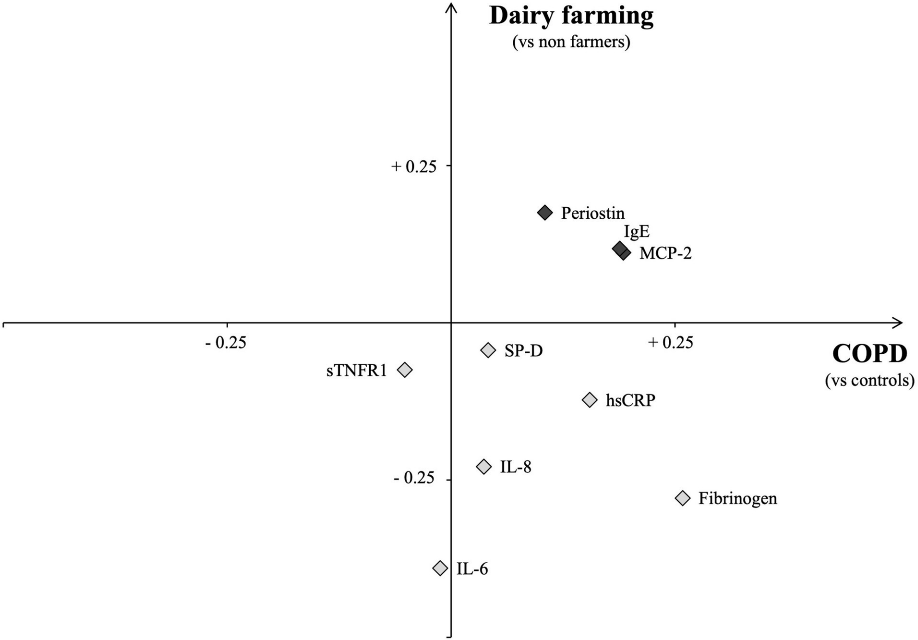
* $p < 0.05$: dairy farmers with COPD *vs* non-farmers with COPD; # $p < 0.05$: dairy farmers with COPD *vs* dairy farmer controls.

Fig. 3. Scatter plot showing the relationship between difference of mean z-score of several cytokines between patients with COPD and controls (X-axis) and dairy farmers and non-farmers (Y-axis).


SCREENING PHASE
Subjects attending screening
n = 8 106
Failed to meet selection criteria (n = 2 985)
Refused to participate (n = 158)
MSA screening program
FEMASAC screening program
Subjects meeting the selection criteria
n = 3 745
Uninterpretable spirometry or technical problems (n = 118)
Subjects with interpretable spirometry
n = 3 627
Subjects with COPD
n = 239
(DF = 191, NF = 48)
Subjects without COPD
n = 3 388
(DF = 2 193, NF = 1 195)
Subjects meeting the selection criteria
n = 1 218
Uninterpretable spirometry or technical problems (n = 47)
Subjects with interpretable spirometry
n = 1 161
Subjects with COPD
n = 116
(NF = 116)
Subjects without COPD
n = 1 045
(NF = 1045)
Refused to participate (n = 138)
Could not be contacted (n = 7)
Excluded after 1st visit (n = 24)
Refused to participate (n = 1 002)
Abnormal spirometry (n = 1 245)
No possible match (n = 1 993)
Excluded after 1st visit (n = 6)
CHARACTERIZATION PHASE
Subjects with COPD
n = 186
Frequency matching
Control subjects
n = 187
Dairy farmers with COPD
n = 101
Non-farmers with COPD
n = 85
Dairy farmer controls
n = 98
Non-farmer controls
n = 89



% of patients
Dairy farmers with COPD
Dairy farmer controls
Non-farmers with COPD
Non-farmer controls
Any comorbidity
High blood pressure
Ischemic heart disease
Arrhythmia
Chronic heart failure
Diabetes
Dyslipidemia
Osteoporosis
Gastro-oesophagal reflux
Obstructive sleep apnea syndrome
Anxiety or depression


Dairy farming
(vs non farmers)
+ 0.25
- 0.25
- 0.25
+ 0.25
COPD
(vs controls)
Periostin
IgE
MCP-2
SP-D
sTNFR1
hsCRP
IL-8
Fibrinogen
IL-6