

Pig farmers have signs of bronchial inflammation and increased numbers of lymphocytes and neutrophils in BAL fluid

B. Pedersen, M. Iversen, B. Bundgaard Larsen, R. Dahl

Pig farmers have signs of bronchial inflammation and increased numbers of lymphocytes and neutrophils in BAL fluid. B. Pedersen, M. Iversen, B. Bundgaard Larsen, R. Dahl. ©ERS Journals Ltd 1996.

ABSTRACT: The purpose of this study was to investigate whether pig farmers had inflammation of the bronchial mucosa and activation of bronchoalveolar lavage (BAL) cells. Pig farmers are exposed to high dust levels and have a prevalence of work-related respiratory symptoms.

Bronchoscopy and BAL were performed in 27 young large-scale pig farmers, who had never smoked. Fifty three lifetime nonsmoking healthy students participated as controls. All farmers and controls had normal lung function (forced expiratory volume in one second (FEV₁) 109 and 105% predicted respectively.

Estimation of macroscopic signs of inflammation in the bronchi (erythema, oedema, secretion and friability) showed that pig farmers had significantly increased signs of inflammation. The median score was 3 (range 0–6) compared to a median score of 0 (range 0–3) in controls. More pig farmers than controls (41 versus 25%) had a positive histamine challenge (provocative dose producing a 20% fall in FEV₁ (PC₂₀) ≤32 mg·mL⁻¹) but the difference was not significant. The cell concentration in BAL fluid was identical in the two groups. Pig farmers had a significantly increased percentage of lymphocytes (median 7, range 1–27 versus median 2, range 0–7) and neutrophils (median 2, range 0–30 versus median 1, range 0–4) compared to controls. Spontaneous migration (19.8 versus 5.5 μm) and chemotaxis (62.6 versus 11.2 μm) was significantly increased in pig farmers compared to controls. After stimulation with zymosan and phorbol myristate acetate (PMA), the reactive oxygen radical generation of purified alveolar macrophages was also significantly increased in pig farmers.

Lifetime nonsmoking pig farmers with normal lung function have macroscopic signs of bronchial inflammation and an increased number of neutrophils in bronchoalveolar lavage. Their alveolar macrophages showed biological signs of activation. The inflammation in pig farmers bronchi may be early signs of bronchitis.

Eur Respir J., 1996, 9, 524–530.

Pig farmers working in large swine-confinement buildings are exposed to high concentrations of respirable dust particles containing feed and faecal particles, dander from pigs, bacteria, fungi, endotoxin, and gases, such as hydrogen sulphide and ammonia [1–3]. After years of pig farming, symptoms of chronic bronchitis [4–7] and obstructive lung disease frequently occur [8–10]. Pig farmers who have work-related lung symptoms are at risk for the development of airways injury [11, 12] and long-term exposure causes inflammatory changes in the airways [13].

In the present study, we investigated whether lifetime nonsmoking pig farmers with normal lung function had inflammation in the airways, estimated by a visual scoring system, and whether cell studies of bronchoalveolar lavage (BAL) showed signs of inflammation and cell activation compared to control persons.

Dept of Respiratory Diseases, University Hospital of Aarhus, Denmark.

Correspondence: R. Dahl
Dept of Respiratory Diseases
University Hospital of Aarhus
Lungeklinikken
Aarhus Kommunehospital
Noerrebrogade 44
DK-8000 Aarhus C
Denmark

Keywords: Alveolar macrophages
bronchial inflammation
bronchoalveolar lavage
neutrophil granulocytes
pig farmers

Received: April 19 1995
Accepted after revision December 6 1995

Methods

Subjects

One hundred and twenty four pig farmers in Aarhus and Vejle counties, Denmark, participated in a clinical investigation [9]. The study population was a random sample of pig farmers, who had been employed in farming for many years on large modern farms each with more than 250 pigs. Lifetime nonsmoking farmers were asked to volunteer to undergo bronchoscopy and bronchoalveolar lavage. Only farmers without a history of atopic disease and with a negative skin-prick test could participate in the bronchoscopy. The personal characteristics of the farmers participating in the bronchoscopy and those who did not are listed in table 1.

Table 1. – Personal characteristics of farmers who underwent bronchoscopy and those who did not

	Farmers with bronchoscopy (n=27)	Farmers without bronchoscopy (n=97)	p-value
Age yrs	39	44	0.03
FEV ₁ % pred	106	100	0.16
FVC % pred	106	100	0.07
FEV ₁ /FVC %	83	81	0.52
Current smokers %	0	25	0.01
Chronic bronchitis %	19	25	0.48
Work-related respiratory symptoms %	30	42	0.24
PC ₂₀ <32 mg·mL ⁻¹ histamine %	41	51	0.34

FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; % pred: percentage of predicted value; PC₂₀: provocative concentration producing a 20% fall in FEV₁.

The controls comprised healthy lifetime nonsmoking medical students, with no exposure to farm environments and no evidence of atopic disease, *i.e.* a negative skin-prick test. No participant had had any bronchial or respiratory tract infection during 8 weeks preceding the examination. All participants had their lung function measured, skin-prick test and histamine challenge performed on a single day. On a separate day, within 3–10 days, bronchoscopy and BAL were performed. Lung function tests and histamine challenge were performed between 9 a.m. and 3 p.m. in farmers and controls. Pig farmers had bronchoscopy performed before noon, after working for 2–3 h in their pig-confinement buildings and until 2–4 h before the procedure. Bronchoscopy was performed at 8.30 a.m. in controls.

All participants gave informed consent after verbal and written information. The study was approved by the Ethics Committee of Aarhus and was performed according to the Declaration of Helsinki.

Questionnaire

All farmers had a structured interview on respiratory symptoms and answered a standardized questionnaire on chronic bronchitis (Medical Research Council (MRC) criteria). Detailed information about working conditions was obtained.

Skin-prick test

Skin-prick tests with nine common allergen extracts: timothy; birch; mugwort; dog; cat; *Dermatophagoides farinae*; *Dermatophagoides pteronyssinus*; Cladosporium; and Alternaria; and three species of storage mites: *Lepidoglyphus destructor*; *Tyrophagus putrescentiae*; and *Acarus siro* were performed in all participants. In pig farmers, skin-prick tests with occupational allergens: cow; swine; and grain were also carried out.

Lung function

Lung function was measured with the Jaeger Tranferscreen II (Erik Jaeger, GmbH, Würzburg, Germany). A noseclip was used during all measurements. The forced expiratory volume in one second (FEV₁) was measured with the subject standing. Tests were performed according to accepted guidelines [14], and all values were expressed as percentage of predicted normal value according to the European values of QUANJER [14].

Histamine challenge

Bronchial reactivity to histamine was tested in accordance with a method described by COCKROFT *et al.* [15]. The aerosol was generated by a Wright nebulizer calibrated to give a constant output of 0.13–0.15 mL·min⁻¹. The aerosol was inhaled during 2 min of tidal breathing through a mouthpiece. A noseclip was used. FEV₁ was measured before the start of the procedure and 30 and 90 s after each inhalation (Vitalograph, Model S; Vitalograph Ltd, Buckingham, UK). FEV₁ after isotonic saline inhalation was used as baseline. Histamine dihydrochloride was inhaled in doubling concentrations from 0.03 to 32 mg·mL⁻¹. The results were expressed as the provocative concentration of histamine producing a 20% fall in FEV₁ (PC₂₀) obtained from the log dose-response curve by linear interpolation of the two last points. Bronchial hyper-reactivity in this study means a PC₂₀ histamine of ≤32 mg·mL⁻¹.

Bronchoscopy and bronchoalveolar lavage

Fibreoptic bronchoscopy and BAL were performed according to recommended guidelines [16]. All bronchoscopies were performed by the same investigator. The patients were premedicated 1 h before bronchoscopy with 10 mg diazepam, orally. Topical anaesthesia with lidocaine was administered in the oro- and hypopharynx, and lidocaine solution 40 mg·mL⁻¹, 4–6 mL, was given in the larynx as a bolus *via* a syringe supplied with a curved needle. The bronchoscope (Olympus BF IT 20, Tokyo, Japan) was inserted transorally *via* a mouthpiece and further local anaesthesia, lidocaine solution 10 mg·mL⁻¹, was given through the bronchoscope. The bronchial mucosa was inspected and airway abnormalities were assessed prior to BAL by the bronchoscopist. The results were quantitated using an inflammatory score. Each of the characteristics: erythema, oedema, secretion and friability, was evaluated by assigning a value 0–3 (0=none; 1=slight; 2=moderate; and 3=severely abnormal) (table 2) [17]. All of the scores were summed to give a total score. The suction channel was washed with sterile isotonic saline immediately before the bronchoscope was wedged into the middle-lobe bronchus. Sterile isotonic saline at 37°C was instilled in three aliquots of 60 mL, aspirated (80–100 cmH₂O), and pooled into a sterile glass bottle on ice to obtain a BAL.

Table 2. – Inflammatory score of the macroscopic signs of bronchial inflammation

	Inflammatory score		p-value
	Pig farmers	Controls	
Erythema	1 (0–2)	0 (0–1)	<0.01
Oedema	1 (0–2)	0 (0–0)	<0.01
Secretions	1 (0–2)	0 (0–0)	<0.01
Friability	1 (0–2)	0 (0–2)	0.03
Total score	3 (0–6)	0 (0–3)	<0.01

Values are presented as median, and range in parenthesis. Significance from Wilcoxon Mann-Whitney rank sum test. Inflammatory score: Erythema: 0=normal, 1=light red, 2=red, 3=beefy red; Oedema: 0=normal, 1=blunting of airway bifurcations, 2=loss of normal airway wall indentations, 3=airway occluded; Secretions: 0=normal, 1=strands of clear mucus, 2=globs of mucus, 3=airway occluded; Friability: 0=normal, 1=punctate submucosal haemorrhage with scope trauma, 2=linear submucosal haemorrhage with scope trauma, 3=frank bleeding with scope trauma. (Inflammatory score system for the macroscopic appearance of the bronchial mucosa. With permission from A.B. Thompson, Section of Pulmonary and Critical Care, Department of Internal Medicine, University of Nebraska, Medical Center, Omaha).

Laboratory techniques

Within 10 min after BAL the lavage fluid was filtered through a nylon web with a pore size of 100 µm (Nytal type 440; Sintab Products AB, Malmö, Sweden) to remove mucus, and the final volume of the BAL was measured. The cells were pelleted by centrifugation at 250×g for 10 min at 4°C. The supernatants were collected and stored in aliquots of 1 and 10 mL at -70°C for later analyses. The cells were washed and resuspended in minimum essential medium (MEM) Dulbecco (Boehringer Mannheim, FRG) with 4 mM glutamine at 4°C. The total number of cells collected was counted in a Bürker-Türk chamber. Cell viability was determined by trypan blue exclusion. Cyto-centrifuge preparations were made using approximately 5×10⁴ cells in 200 µL medium and centrifugation at 90×g for 5 min (Shandon Cytospin 2; Southern Products Ltd, Cheshire, UK). The preparations were stained with May-Grünwald Giesma reagents, and the differential cell counts were performed by counting at least 600 cells on each of two preparations.

Purification and isolation of alveolar macrophages from BAL fluid

Alveolar macrophages (AM) of more than 98% purity were isolated from the BAL fluid by a discontinuous metrizamide gradient (Nyegaard, Oslo, Norway) according to a modification of the method described by VADAS *et al.* [18]. The gradient consisted of 2.0 mL layers of 18, 20, 25, 26 and 27% metrizamide made from a stock solution of 30% metrizamide in Tyrode's solution, containing 0.1% gelatine (Merck, Darmstadt, FRG) to give the following densities: 1.082, 1.089, 1.106, 1.109, 1.113

g·mL⁻¹. The gradient was prepared in a polypropylene tube at 4°C. The cells were resuspended in 1 mL MEM Dulbecco and placed on top of the gradient. The gradient was centrifuged for 45 min, at a 45° angle, 1,200×g, room temperature. Each cell fraction was collected from the top of the gradient and washed in 0.9% w/v saline. The cells were resuspended in Gey's buffer and the cell number was counted in a Bürker-Türk chamber after staining with trypan blue to calculate cell viability. Cyto-centrifuged preparations were prepared for cell differential count. The AMs obtained from the lowest density fraction of the gradient were resuspended at a concentration of 1×10⁶ cells·mL⁻¹ for the functional analyses.

Chemiluminescence assay

The oxidative metabolism of the purified AMs was determined by chemiluminescence during adherence and phagocytosis of serum-opsonized zymosan particles (Sigma Chemical Corp., St. Louis, MO, USA) (STZ). The AMs were added to a suspension of zymosan particles of 4 mg·mL⁻¹ in Tris-buffer and direct stimulation of the membrane was done by adding phorbol-12-myristate-13-acetate (PMA) (Sigma Chemical Corp., St. Louis, MO, USA) dissolved in dimethyl sulphoxide (DMSO) (Sigma Chemical Corp., St. Louis, MO, USA) and diluted in Tris-buffer to a concentration of 1 mM. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Boehringer Mannheim, FRG) dissolved in DMSO and diluted in Tris-buffer to a concentration of 25 µg·mL⁻¹ was used to amplify chemiluminescence. Chemiluminescence was measured with a Bioluminat cell tester (Bioluminat LB 9500; Bertholdt, FRG). One hundred microlitres of the AM cell suspension was placed in a cuvette at 37°C and 100 µL luminol and 100 µL STZ or PMA were added. The samples were mixed and the light emission was registered at intervals of 2 s. The registration was followed until the maximum light emission had been obtained.

Spontaneous migration and chemotactic activity of AMs

Migration of AMs was assessed by the leading-front technique, using a modified Boyden chamber. The filters (Millipore Corp., Molsheim, France) had a pore size of 8 µm and were 150 µm thick. AMs were suspended in Gey's buffer containing 0.2% albumin. One hundred microlitres of the cell suspension containing 1×10⁶ AMs·mL⁻¹ was added to the upper well. Spontaneous migration was defined as the migrating distance with Gey's buffer below the filter. The chemotaxis was defined as the migration of AMs towards 10% complement activated serum in Gey's buffer below the filter. The AMs were allowed to migrate for 3 h at 37°C. The micropore filters were removed and fixed in 96% ethanol. The filters were stained with Mayer's haemalaun staining solution (Merck, Darmstadt, FRG) and air-dried. The filters were then mounted on glass slides. The spontaneous migration and chemotaxis of AMs were quantified by measuring the

longest distance in μm that the AM had migrated in each filter using a light microscope. At least three measurements were made from each duplicate filter and the results were expressed as a mean.

Statistics

Statistical evaluation was performed with the Statistical Package for the Social Sciences (SPSS) [19]. Variables which showed a normal distribution were expressed as mean \pm SD, and comparison of means for parametric values was made by analyses of variance. Variables with a skewed distribution or censored values were expressed as median \pm range and nonparametric statistics, Wilcoxon Mann-Whitney rank sum test was used for analyses. The chi-squared test was used for comparing dichotomous variables. Logistic regression analysis was used to evaluate predictors for bronchial hyperreactivity. Probability values equal to or less than 0.05 were considered to be statistically significant.

Results

Personal characteristics

Twenty seven lifetime nonsmoking pig farmers (2 females and 25 males), mean age 38 yrs (SD \pm 11 yrs) agreed to participate in the study. They had worked on farms for 16 yrs (range 4–45 years) and had been working as pig farmers for 11 yrs (range 2–30 yrs) on average. Their working task was pig farming exclusively and they were all large-scale pig farmers with, on average, 800 pigs (range 250–1,500 pigs). The farmers spent on average 5 h (range 2–9 h) each day, 7 days a week, in the pig-confinement buildings. Five pig farmers had symptoms of chronic bronchitis (MRC criteria) with a median duration of 7 yrs (range 2–15 years). Symptoms were, however, mild and no farmer had sought medical attention for bronchitis. No farmer had a diagnosis of current or earlier asthma. Eight farmers had work-related respiratory symptoms, such as shortness of breath, wheezing or dry cough, of whom four had chronic bronchitis. There was no significant difference with respect to age, lung function, PC₂₀ histamine, inflammatory score by bronchoscopy, cell differential count in BAL, and chemotactic activity and chemiluminescence of AMs between farmers with respiratory symptoms (chronic bronchitis or work-related respiratory symptoms, n=9) and farmers without respiratory symptoms.

The control group consisted of 53 (25 females and 28 males) healthy lifetime nonsmoking: students with a mean age of 24 yrs (SD \pm 2.4 yrs). No control person fulfilled MRC criteria for chronic bronchitis. The controls were significantly younger than the pig farmers ($p<0.01$).

Lung function and histamine reactivity

Mean FEV₁ percentage predicted showed no significant difference between the groups (pig farmers 109%

(SD \pm 11%); controls 105% (SD \pm 11%)). Histamine PC₂₀ was lower but not significantly so ($p=0.08$) in pig farmers compared to controls (fig. 1). A PC₂₀ value ≤ 32 mg \cdot mL⁻¹ was found in 41% of pig farmers and 25% of the control group. A logistic regression analysis with PC₂₀ as dependent variable showed that the inflammatory score (≤ 3 , >3) was a significant explanatory variable for the presence of bronchial hyperreactivity ($p=0.03$), whereas FEV₁ percentage predicted (≤ 105 , >105 ; $p=0.14$) and percentage neutrophils in BAL (≤ 2 , >2 ; $p=0.16$) were not.

Macroscopic appearance of bronchial mucosa

The median value for each of the inflammatory scores, erythema, oedema, secretion and friability were significantly higher in the pig farmers compared to controls (table 2), and when a total score was calculated this was significantly higher in pig farmers compared to controls ($p<0.01$). Macroscopic signs of an inflamed mucosa (inflammatory score >3) in pig farmers was not significantly associated with the presence of respiratory symptoms ($p=0.34$). There was a significant association between percentage neutrophils in BAL and inflammatory score ($r=0.47$; $p=0.01$).

Lavage fluid recovery, cell number and cell differential

In all subjects a total of 180 mL isotonic saline was instilled and the recovery of fluid was similar in pig farmers and controls.

The total cell number was significantly increased ($p<0.01$) in controls ($11.9\pm 4.6\times 10^6$) compared to pig farmers ($7.2\pm 5.7\times 10^6$), but cell concentration was not different between the two groups (fig. 2). The mean recovery was 67% in controls and 52% in pig farmers.

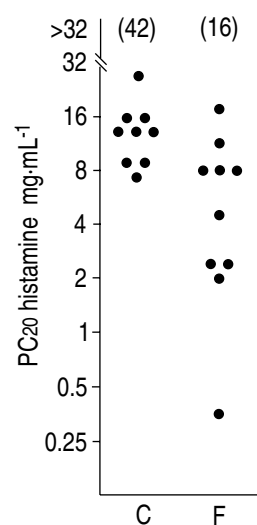


Fig. 1. – Distribution of PC₂₀ histamine in controls (C) and pig farmers (F). Numbers in brackets are persons with PC₂₀ >32 mg \cdot mL⁻¹. PC₂₀: provocative concentration producing a 20% fall in forced expiratory volume in one second.

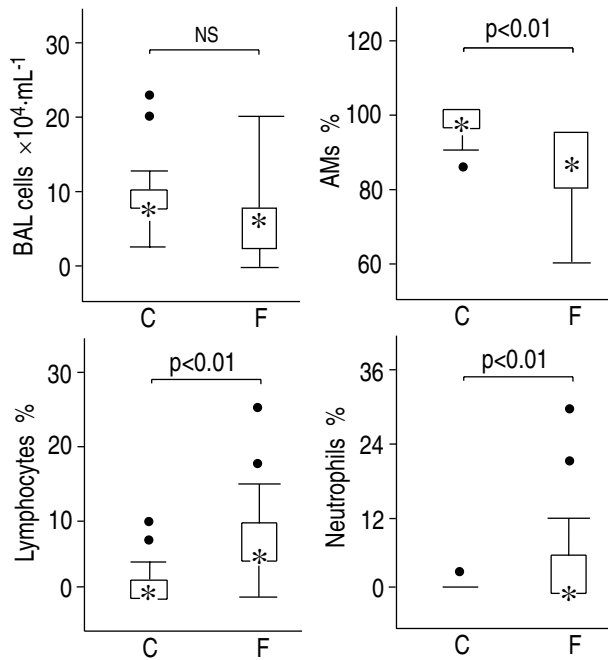


Fig. 2. – Cell number in aspirated BAL and cell differentiated counts in BAL fluids. The box represents 50% of the observations, the asterisk the median value and the bars the 95% percentile. Observations outside the 95% interval are shown with dots. C: controls; F: pig farmers; BAL: bronchoalveolar lavage fluid. AMs: alveolar macrophages.

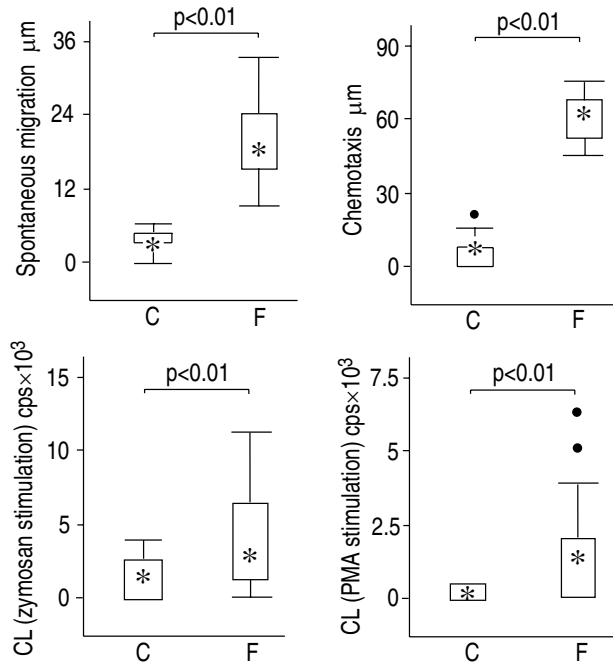


Fig. 3. – Chemotactic activity and spontaneous migration of purified AMs and oxidative metabolism of purified AMs measured by chemiluminescence after stimulation with zymosan and PMA. The box represents 50% of the observations, the asterisk the median value, and the bars the 95% percentile. Observations outside the 95% interval are shown with dots. C: controls; F: farmers; AMs: alveolar macrophages; PMA: phorbol myristate acetate; cps: counts per second. CL: chemiluminescence.

Cell differential counts showed a significantly ($p<0.01$) increased percentage of lymphocytes (median 7%, range 1–27%; and median 2%; range 0–7%, respectively), and

neutrophils (median 2%, range 0–30%; and median 1%, range 0–4%, respectively) in pig farmers compared to normal controls. This was concomitant with a significantly lower percentage of AMs (fig. 2) in pig farmers (median 89%, range 64–99%) compared to controls (median 97%, range 85–99.9%).

Spontaneous migration and chemotaxis were significantly increased ($p<0.01$) in pig farmers compared to controls (spontaneous migration 19.83 and 5.46 μm ; and chemotaxis 62.63 μm and 11.26 μm) (fig. 3).

The oxidative metabolism of purified AMs after stimulation with zymosan and PMA showed a significant increase ($p<0.01$) in light emission in pig farmers (zymosan 4,311 counts per second (cps); and PMA 1,830 cps) compared to controls (zymosan 1,959 cps; and PMA 130 cps) (fig. 3). There was no significant difference in oxidative metabolism with zymosan and PMA between samples purified to 98, 99 or 100% AMs ($p>0.05$).

Discussion

In this study, we found that lifetime nonsmoking large-scale pig farmers with normal lung function had macroscopic signs of inflammation in the bronchial mucosa visible by bronchoscopy. Furthermore, BAL cell differential counts showed increased percentages of lymphocytes and neutrophils in pig farmers, and their macrophages had increased chemotactic activity and oxidative metabolism. Five farmers had mild symptoms of chronic bronchitis, and a total of nine farmers had respiratory symptoms. Comparisons were performed with these persons excluded from the analysis but this did not change results.

Farmers and controls were selected according to the same criteria. To exclude the effects of smoking and asthma on the airways, only lifetime nonsmokers and persons with a negative skin-prick test and no asthma were accepted. The controls were significantly younger than the farmers but this was not thought to invalidate the results.

All pig farmers in this study had normal FEV₁ and the mean FEV₁ was slightly higher than in controls, whereas earlier studies [5, 9] have shown that pig farmers have somewhat lower FEV₁ than comparable farmers with other exposures. This difference is probably caused by the specific selection of lifetime nonsmoking subjects in our study. The farmers who underwent bronchoscopy were significantly younger and tended to have higher lung function and fewer respiratory symptoms than those without bronchoscopy. The farmers who underwent bronchoscopy possibly represent a more healthy group of farmers than the average pig farmer but this does not weaken the conclusions from this study.

The results of the study, from which the farmers in the present study were recruited, suggested that pig farmers had more bronchial reactivity than dairy farmers [9], and two other studies have independently found that pig farmers had more bronchial reactivity than other farmers [12, 20]. This strongly indicates the presence of substances in pig-confinement buildings that promote changes in the airways and influence bronchial reactivity. In healthy nonsmoking urban subjects, exposure for 5–9 h

in a swine-confinement building resulted in increased bronchial reactivity [21], and an intense inflammatory response with neutrophilia and increased concentrations of albumin and fibronectin in BAL fluid [22]. This supports the hypothesis that working in pig-confinement buildings causes bronchial hyperreactivity by the inhalation of irritant dust. It has yet to be determined whether the mechanism is related to specific mediators or cells taking part in the inflammatory response, or is caused by inflammation associated with mucosal oedema in the airways. In smokers, bronchial reactivity has been associated with bronchiolar inflammation [23], and even a moderate increase in wall-thickness in the bronchioles, caused by oedema, cellular infiltration and hyperaemia, can result in increased response to bronchoconstrictive agents, such as histamine [24]. This mechanism has been demonstrated to be of importance in asthmatics and probably also in patients with chronic airways obstruction [25].

In previous bronchoscopy studies [12, 13] in pig farmers, the macroscopic appearance of the bronchial mucosae was not evaluated. By inspection of the bronchial mucosa, we were surprised to find clear macroscopic abnormalities in these pig farmers because they were lifetime non-smokers and only five had symptoms of a mild chronic bronchitis. The investigator was not blinded and this may possibly have affected the results. The difference between the two groups was, however, pronounced and not subtle and we do not think that the unblinded approach represents a critical bias. In asthmatic patients, a weak correlation between airway inflammation evaluated by visual inspection and the clinical severity by the Aas score was recently described [26]. In our study, there was a correlation between the percentage of neutrophils in BAL and the visual inflammation score. So far, the visual score has not been correlated to the degree of inflammation assessed by histology. Studies are needed to evaluate the clinical relevance of the macroscopic changes found by bronchoscopy and the prognostic implication.

In controls, the total number of BAL cells was increased compared to pig farmers, but no difference was found in cell concentration or recovery. The cell differential counts showed a significant increase in the percentage of lymphocytes and neutrophils in pig farmers compared to controls. The findings are similar to those of LARSSON *et al.* [13]. There seems to be a discrepancy between chronic exposure accompanied by a moderate increase in BAL neutrophils, and acute exposure where neutrophils are increased 75 fold [22]. The changes following acute exposure are similar to those produced by inhalation challenge with endotoxin [27], or grain dust extract [28]. The macrophages in our pig farmers showed increased activity, and the functional studies revealed increased chemotactic activity and increased oxygen radical formation after stimulation. Similar observations have been made in various diseases, leading to decreased lung function and apparently an expression of an active inflammatory process regardless of the initiating mechanism. BAL cells from smokers [29], sarcoid patients [30], interstitial lung disease [31], and bronchial asthma [32] show

increased oxygen radical formation. The direct injury to tissue or the inactivation of alpha₁-antitrypsin by reactive oxygen [33] may partly determine the loss of lung function and also explain the increased occurrence of obstructive lung disease in pig farmers after many years of exposure. In bronchial asthma, KELLY *et al.* [34] found an inverse correlation between the concentration of methacholine that gave a 20% fall in FEV₁ and the oxygen radical formation by AMs; thus relating the activity of an inflammatory cell to a clinical measure. It is of interest that the changes observed in the bronchi of pig farmers were not associated with symptoms of chronic bronchitis or work-related respiratory symptoms. This makes it very difficult on clinical grounds to evaluate possible prognostic factors or persons at risk for development of permanent lung damage. The number of pig farmers with respiratory symptoms was, however, small.

No environmental measurements of dust concentrations were undertaken at the pig farms in our study, but it is very well-documented that pig farming is always associated with a high dust exposure and all available evidence points to the dust exposure as the cause of the high frequency of respiratory symptoms in pig farmers compared to other farmers or controls [2]. Dust measurements from farms similar to those included in this study and from the same counties have shown levels equivalent to those reported from other countries [35]. Although the farmers studied were young and had normal lung function, their airways were altered with inflammatory changes and possibly bronchial hyperreactivity. The relevance of the inflammatory changes with regard to the development of respiratory symptoms and airways obstruction is yet to be established. Cross-sectional studies [9, 10], and one longitudinal study [36], suggest that pig farming is associated with an accelerated decline in lung function and a study with biopsies of the airways [12] demonstrated that swine-confinement workers had morphological changes in their airways, with thickening of the epithelial basement membrane. The most extensive follow-up study of pig farmers until now [37] demonstrated that pig farmers had slightly lower lung function than control farmers and that this difference persisted throughout the study. The mean follow-up-time was, however, only 2 yrs. Pig farmers had larger across-workshift changes in lung function than control farmers in the follow-up period [37]. The changes in lung function in pig farmers observed in various studies might be caused by the inflammation observed in their airways.

References

1. Donham KJ, Scallon LJ, Pependorf W, Freuhaft MW, Roberts RC. Characterization of dusts collected from swine-confinement buildings. *Am Ind Hyg Assoc J* 1986; 47: 404-410.
2. Rylander R, Donham KJ, Hjort C, Brouwer R, Heederik D. Effects of exposure to dust in swine-confinement buildings: a working group report. *Scand J Work Environ Health* 1989; 15: 309-312.
3. Donham KJ, Haglund P, Peterson Y, Rylander R, Belin L. Environmental and health studies in farm workers in

- Swedish swine-confinement buildings. *Br J Ind Med* 1989; 46: 31–37.
4. Donham KJ, Zavala DC, Merchant JA. Respiratory symptoms and lung function among workers in swine confinement buildings. *Arch Environ Health* 1984; 39: 96–101.
 5. Donham KJ. Health effects from work in swine-confinement buildings. *Am J Ind Med* 1990; 17: 17–25.
 6. Iversen M, Dahl R, Korsgaard J, Hallas T, Juel Jensen E. Respiratory symptoms in Danish farmers: an epidemiological study of risk factors. *Thorax* 1988; 43: 872–877.
 7. Holness DL, O'Blenis EL, Sass-Kortsak A, Pilger C, Nethercott JR. Respiratory effects and dust exposures in hog-confinement buildings. *Am J Ind Med* 1987; 11: 571–580.
 8. Iversen M, Dahl R, Jensen EJ, Korsgaard J, Hallas T. Lung function and bronchial reactivity in farmers. *Thorax* 1989; 44: 645–649.
 9. Iversen M, Pedersen B. Relation between respiratory symptoms, type of farming, and lung function disorders in farmers. *Thorax* 1990; 45: 919–923.
 10. Dosman JA, Graham BL, Hall DL, *et al.* Respiratory symptoms and alterations in pulmonary function tests in swine producers in Saskatchewan: results of a survey of farmers. *J Occup Med* 1988; 30: 715–720.
 11. Cormier Y, Boulet LP, Bedard G, Tremblay G. Respiratory health of workers exposed to swine-confinement buildings only or to both swine confinement buildings and dairy farms. *Scand J Work Environ Health* 1991; 17: 269–275.
 12. Schwartz DA, Landas SK, Lassise DR, Burmeister LF, Hunninghake GW, Merchant JA. Airway injury in swine-confinement workers. *Ann Intern Med* 1992; 116: 630–635.
 13. Larsson K, Eklund A, Malmberg P, Belin L. Alterations in bronchoalveolar lavage fluid but not in lung function and bronchial responsiveness in swine-confinement workers. *Chest* 1992; 101: 767–774.
 14. Quanjer PhH. Report Working Party. Standardization of lung function tests. European Coal and Steel Community. *Eur Respir J* 1993; 6 (Suppl. 16) 7–29.
 15. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; 7: 235–243.
 16. Workshop summary and guidelines. Investigational use of bronchoscopy, lavage and bronchial biopsies in asthma and other airway diseases. *J Allergy Clin Immunol* 1991; 88: 808–814.
 17. Thompson AB, Daughton D, Robbins RA, Ghafouri MA, Oehlerking M, Rennard SI. Intraluminal airway inflammation in chronic bronchitis: characterization and correlation with clinical parameters. *Am Rev Respir Dis* 1989; 140: 1527–1537.
 18. Vadas MA, David JR, Butterworth A, Pisani NT, Siongok TA. A new method for purification of human eosinophils and neutrophils, and a comparison of the ability of these cells to damage schistosomula of *Schistosoma mansoni*. *J Immunol* 1979; 122: 1228–1236.
 19. Norusis MJ. SPSS/PC+4 Base Manual, 4.0 Statistics Manual, and 4.0 Advanced Statistics Manual. SPSS Inc., 1990, Chicago.
 20. Zhou C, Hurst TS, Cockcroft DW, Dosman JA. Increased airways responsiveness in swine farmers. *Chest* 1991; 99: 941–944.
 21. Malmberg P, Larsson K. Acute exposure to swine dust causes bronchial hyperresponsiveness in healthy subjects. *Eur Respir J* 1993; 6: 400–404.
 22. Larsson KA, Eklund AG, Hansson L-O, Isaksson B-M, Malmberg PO. Swine dust causes intense airways inflammation in healthy subjects. *Am J Respir Crit Care Med* 1994; 150: 973–977.
 23. Mullen JB, Wiggs BR, Wright JL, Hogg JC, Paré PD. Nonspecific airway reactivity in cigarette smokers. *Am Rev Respir Dis* 1986; 133: 120–125.
 24. Moreno RH, Hogg JC, Paré PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986; 133: 1171–1180.
 25. Matsuba K, Wright JL, Wiggs BR, Paré PD, Hogg JC. The changes in airways structure associated with reduced forced expiratory flow in one second. *Eur Respir J* 1989; 2: 834–839.
 26. Van Vyve Th, Chanez P, Lacoste JY, Bousquet J, Michel FB, Godard P. Assessment of airway inflammation in asthmatic patients by visual endoscopic scoring system. *Eur Respir J* 1993; 6: 1116–1121.
 27. Sandström T, Bjermer L, Rylander R. Lipopolysaccharide: (LPS) inhalation in healthy subjects increases neutrophils, lymphocytes, and fibronectin levels in bronchoalveolar lavage fluid. *Eur Respir J* 1992; 5: 992–996.
 28. Von Essen SG, Robbins RA, Thompson AB, Ertl RJ, Linder J, Rennard S. Mechanisms of neutrophil recruitment to the lung by grain dust exposure. *Am Rev Respir Dis* 1988; 138: 921–927.
 29. Bergstrand H, Björnson A, Blaschke E, *et al.* Effects of an inhaled corticosteroid budesonide, on alveolar macrophage function in smokers. *Thorax* 1990; 45: 362–368.
 30. Fels AOS, Nathan CF, Cohn ZA. Hydrogen peroxide release by alveolar macrophages from sarcoid patients and by alveolar macrophages from normals after exposure to recombinant interferon alpha, beta, and gamma and 1,25 dihydroxyvitamin D3. *J Clin Invest* 1987; 80: 381–386.
 31. Clement A, Chadelat K, Masliah J, *et al.* A controlled study of oxygen metabolite release by alveolar macrophages from children with interstitial lung disease. *Am Rev Respir Dis* 1987; 136: 1424–1428.
 32. Cluzel M, Damon M, Chanez P, *et al.* Enhanced alveolar cell luminol-dependent chemiluminescence in asthma. *J Allergy Clin Immunol* 1987; 80: 195–201.
 33. Hubbard RC, Ogushi F, Fells GA, *et al.* Oxidants spontaneously released by alveolar macrophages of cigarette smokers can inactivate the active site of alpha₁-antitrypsin, rendering it ineffective as an inhibitor of neutral elastase. *J Clin Invest* 1987; 80: 1289–1295.
 34. Kelly C, Ward C, Stenton CS, Bird G, Hendrick DJ, Walters EH. Number and activity of inflammatory cells in bronchoalveolar lavage fluid in asthma and their relation to airway responsiveness. *Thorax* 1988; 43: 684–692.
 35. Takai H, Larsen E, Pedersen S. Støv og støvmåling i svinestalde II. Orientering nr. 43. (Dust measurements in pig-confinement buildings. In Danish). State Institute of Agricultural Engineering, Horsens, Denmark, 1986.
 36. Iversen M, Brink O, Dahl R. Lung function in a five year follow-up study of farmers. *Ann Agric Environ Med* 1994; 1: 39–43.
 37. Schwartz DA, Donham KJ, Olenchock SA, *et al.* Determinants of longitudinal changes in spirometric function among swine confinement operators and farmers. *Am J Respir Crit Care Med* 1995; 151: 47–53.