Immunological and Respiratory Findings in Swine Farmers

Eugenija Zuskin, Bozica Kanceljak, E. Neil Schachter, Jadranka Mustajbegovic, Satindra Goswami, Saul Maayani, Zvi Marom, and Nicholas Rienzi

Andrija Stampar School of Public Health, Zagreb, Yugoslavia; and Mount Sinai School of Medicine, New York, New York

The prevalence of respiratory symptoms and ventilatory capacity abnormalities in relation to immunological status was studied in 32 swine farmers and in 39 controls. A large number of swine farmers reacted to swine confinement building antigens (swine hair, 34%, swine confinement agents, 28%) but also to other extracts such as animal food (78%) and corn flour (37%). Control workers also reacted to these antigens in similar frequencies. Increased serum IgE levels were found in 3 swine farmers (9.4%) and all 3 had positive skin tests to at least one of the swine antigens. Among control workers one (2.6%) had an increased serum IgE level; this worker exhibited a positive skin reaction to swine food antigen. Swine farmers with positive skin reactions had across-shift reductions of FEF50 and FEF25 significantly larger than those with negative skin tests ($P < 0.01$). Preshift measured ventilatory capacity data (FEV1, FEF50, FEF25) in swine farmers with positive skin tests were significantly lower (compared to predicted) than in those with negative skin tests. Additionally, we showed that a water-soluble swine confinement building antigen causes a dose-related contraction of nonsensitized guinea pig trachea smooth muscle studied *in vitro*. Our data indicate significant differences in lung function between swine workers with positive and negative skin tests. We suggest that skin testing may be helpful in identifying workers at risk for developing lung disease. © 1991 Academic Press, Inc.

INTRODUCTION

Numerous airborne pollutants in the environment of pig farm buildings are recognized as potentially hazardous to the respiratory tract. These pollutants include mixtures of substances containing components of animal and plant origin, as well as microbial constituents and different gases. A number of investigators have studied environmental antigens that are related to animals and might be involved in the pathogenesis of lung disease among swine farmers. In a study by Brouwer *et al.* (1986) no workers had IgE antibodies against pig-derived antigen extracts (urine and hair). In one individual, elevated IgE antibody levels against the feed components, wheat, tapioca, corn, and soy, were found. However, the group of "closed" farmers (breeding and fattening pigs) had higher IgG4 levels against pig-derived antigens. Katila *et al.* (1981), in a Finnish study, reported precipitins against swine antigens in 10% of swine workers and increased IgG4 level in 30% of swine workers. No IgE antibodies were found. In a Dutch study (Brouwer *et al.*, 1990), 13% of 130 pig farmers had elevated levels of IgE immunoglobulins against storage mites. No IgE antibodies were found against pig-derived antigens. The authors found elevated IgG4 levels against pig urine and pig

120

hair. The relationship between total dust level and IgG4 serum antibody levels against pig-derived antigens was demonstrated only in nonsmokers. In another study of Donham (1990) IgG antibodies to swine house antigens were common; no relation to symptoms could be found. Matson *et al.* (1983) found that among 41 hog farmers, elevated IgG antibody levels to hog barn dust and/or hog feed in 7 (17.1%) individuals were present; two of these sera contained precipitating antibodies to hog barn dust and hog feed. Elevated IgE antibody levels to hog pelt, hog urine, and hog serum were found in only one (0.2%) individual. However, skin tests with these materials were negative. In no case could the presence of IgE or IgG antibodies be correlated with symptoms. Occupational asthma caused by allergy to pig's urine with increased serum levels of specific IgE antibody to the urine extract was reported by Harries and Cromwell (1982). Rylander *et al.* (1990) described different effects of exposure to dust in swine confinement buildings and reported that there was no evidence that antigen–antibody reactions were important in the pathogenesis of pulmonary diseases in pig farmers.

The present study is an extension of a previous one carried out in a group of swine farmers in Yugoslavia. In the current study we investigated the frequency of respiratory symptoms and ventilatory capacity changes in relation to sensitization against swine confinement building antigens in swine farmers. In addition, we evaluated *in vitro* the possible constrictive effect of an extract derived from swine confinement building dust on isolated guinea pig tracheal smooth muscle.

SUBJECTS AND METHODS

1. Immunological Study

Subjects. The study was performed on 32 male workers in one swine farm in Yugoslavia. They represented 85% of all workers on the farm. Their mean age was 35 years (range: 19–58 years), mean height 168 cm (range: 158–186 cm), and mean duration of exposure 8 years (range: 1–20 years). Ninety percent of the studied workers were regular smokers, smoking on the average 20 cigarettes daily. In addition, a control group of 39 male workers of similar age, smoking habit, and duration of employment was studied as a control for the immunological investigation. They were employed packing chicken meat in a food-producing industry with no exposure to noxious dust, fumes, or gases.

Immunological study. Skin prick tests were performed in 32 swine farmers and in 39 control workers. Extracts were prepared from the material collected in the swine confinement building such as swine food (which is a mixture of wheat, corn, soy, and fish flour), corn flour, swine hair, and floor material (swine confinement agents). A standard immunological technique was used for preparing occupational allergens (Sheldon *et al.*, 1967). In addition workers were skin-tested with histamine base (0.01 mg/ml), bacteria, mold, *Dermatophagoides pteronyssinus* antigens, and a buffer solution as a control solution. Bacterial antigen was prepared from cultures of *Haemophilus influenzae, Streptococcus pneumoniae, Streptomyces viridans, Streptococcus pyogenes,* and *Staphylococcus aureus* in a concentration of 60×10^6/ml. Mold antigen was a mixture of *Alternaria, Penicillium, Mucor, Cladosporium, Aspergillus niger,* and *Aspergillus fumigatus* in a 0.2%

solution. Skin reactions were read after 20 min. Skin prick test were considered positive if the diameter of the observed wheal was >3 mm.

The serum level of total IgE immunoglobulin was determined by a reference laboratory PRIST (Pharmacia Diagnostics AB, Upsala, Sweden) using the direct radioimmunologic "sandwich" technique. Levels of IgE below 125 IU/ml were considered normal.

Respiratory symptoms. Chronic respiratory symptoms were recorded in swine farmers using the British Medical Research Council Committee questionnaire on respiratory symptoms (1960) with additional questions on occupational asthma (WHO, 1986). For all workers a detailed occupational history as well as questions about their smoking habit was recorded. The following definitions were used:

Chronic cough or phlegm: cough and/or phlegm production for at least 3 months per year;

Chronic bronchitis: cough and phlegm for a minimum of 3 months a year and for not less than 2 successive years;

Dyspnea grades: grade 3, shortness of breath when walking with other people at an ordinary pace on level ground; grade 4, shortness of breath when walking at their own pace on level ground.

Occupational asthma: recurring attacks of dyspnea, chest tightness, and pulmonary function impairment of the obstructive type diagnosed by physical examination and spirometric measurements during exposure to dust at or following work.

In all swine farmers the presence of acute symptoms during the work shift such as cough, dyspnea, chest tightness, irritation or dryness of the throat, secretion, dryness or bleeding of the nose, eye irritation, and headache were specifically recorded.

Ventilatory capacity. Ventilatory capacity was measured by recording maximum expiratory flow-volume (MEFV) curves using a portable flow-volume spirometer (Pneumoscreen, Jaeger, Germany). Measurements were performed on the first day of the week (Monday) before (6 am) and after the work shift (2 pm). The forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV1), and maximum flow rates at 50% and the last 25% of the control vital capacity were measured on these curves. At least three MEFV curves were recorded and the best value was used as the result of the test. The measured Monday preshift values of ventilatory capacity were compared with the expected normal values of Quanjer (1983).

Statistical analysis. The results of ventilatory capacity measurements were analyzed by using the t test for differences of paired (acute effects across shift) and unpaired (comparing baseline to predicted values) variables. The χ^2 test was used for testing differences in the prevalence of respiratory symptoms. $P < 0.05$ was considered statistically significant.

2. Swine Confinement Building Antigen Assay in Vitro

In order to investigate the potential of swine confinement building dust to provoke airway smooth muscle contraction we tested the bronchoconstricting potential of extracts of swine confinement building antigen, which is derived from

a mixture of swine hair, swine food, and floor material (swine confinement building antigen), on guinea pig trachea. The extract was prepared in a standard way as described by Sheldon et al. (1967) in a concentration 1:10 w/v.

We used the trachea of young albino Hartley male guinea pigs (300–390 g) purchased from Perfection Breeders, PA. Animals were sacrificed by CO_2 asphyxiation for 2 min and the trachea was removed within 3 min. The animal tissues were manually trimmed of excess fat and connective tissue. Three segments ("rings," each 4–6 mm wide) were cut from a single trachea, and each was suggested between two L-shaped stainless-steel hooks mounted in a 20-ml organ chamber containing Krebs–Hanseliet buffer of the following composition (μM): NaCl, 110.0; KCl, 4.80; $CaCl_2$, 2.35; $MgSO_4$, 1.20; KH_2PO_4, 1.20; $NaHCO_3$, 25.0; dextrose, 11.0; and Na_2EDTA, 0.03, in glass-distilled water. Organ chambers were maintained at 36.5 ± 0.5°C and were continuously aerated with 95% O_2 and 5% CO_2 to maintain pH 7.5 ± 0.1. The tissue segments were initially set to 2 g of tension and were allowed to relax for approximately 1.5 hr before the experiment began. During that period the tissue was washed at 15-min intervals. After the relaxation period, the tension in each tissue segment was adjusted to 2 g for all subsequent assays. Isometric contractions were recorded using Grass FT03C displacement transducer attached to a Grass polygraph recorder. Before and after, concentration–response assays with our extracts of pig-derived antigen were performed, a challenge with carbachol 10^{-5}M was run. A dose–response curve with pig-derived antigen was obtained by adding progressively increasing volumes of each extract or krebs (used as a control) into the tissue bath in progressive aliquots of 10, 30, 100, 300, and 1000 μl. The potency of the extract was determined by comparing the biological activity with the maximal contraction induced by carbachol (10^{-5}M) on the same tissue. The data are expressed as a percentage of the initial maximal carbachol concentration (10^{-5}M).

The protein content in the swine confinement building antigen was determined by the Lowry method (Lowry et al., 1951).

RESULTS

1. Immunological Study

Data on skin tests to different swine confinement building allergens in swine farmers and in control workers are presented in Fig. 1. In the total group of 32 swine farmers, 14 (43.8%) demonstrated positive skin reaction to one of the swine antigens (swine confinement agents or swine hair). A similar number of control workers reacted to these swine antigens (15; 38.5%). The largest number of positive reactions among swine farmers were to swine food (78%), followed by corn flour (37%), swine hair (34%), D. pteronyssinus, and swine confinement agents (28%). Considerably fewer of the swine workers reacted to bacteria and molds extracts (9%). None reacted to the buffer solution. Among control workers the largest number reacted to swine food (51%), followed by corn flour and swine confinement agents (25%), D. pteronyssinus (20%), swine hair (17%), bacteria (4%), and molds (3%). There were no significant differences in the prevalence of positive skin reactions between swine farmers and control workers for any of the
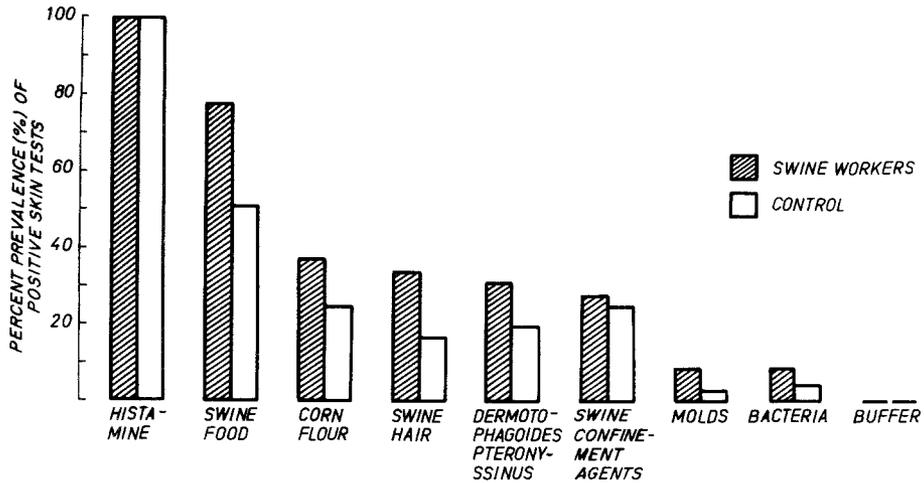
FIG. 1. Prevalence of positive skin prick test to different swine confinement building allergens in swine farmers and in control workers.

tested extracts ($P > 0.05$). Three swine farmers (9.4%) and one control worker (2.6%) had increased IgE serum level (NS). Two swine farmers among the three with increased IgE serum level demonstrated positive skin reaction to swine food, corn flour, and swine hair and the remaining worker to swine confinement agents. One control worker with an increased IgE serum level had positive skin reaction to swine food.

Respiratory symptoms. Table 1 presents the prevalence of chronic respiratory symptoms. The group of swine workers as a whole complained of respiratory symptoms more frequently than did controls. This was significant only for chest tightness. Workers with positive skin tests had a higher prevalence of chronic cough, chronic phlegm, and chronic bronchitis than workers with negative skin tests; however, the differences were not statistically significant ($P > 0.05$). There were no workers with occupational asthma, nasal catarrh, or sinusitis.

Table 2 presents the prevalence of acute symptoms in swine farmers during the work shift by skin tests results. A large number of farmers complained of acute symptoms being particularly pronounced for cough, dyspnea, dryness or burning of the throat and eye burning. In general, symptoms were equally prevalent in workers with positive and negative skin tests.

Ventilatory capacity. The data on ventilatory capacity are presented in Table 3 separately for swine farmers with positive and negative skin tests. Significant acute reductions were recorded for all tests except for FVC in farmers with negative skin tests. Larger across-shift reductions were recorded in farmers with positive skin tests (FEF50, -10.6%; FEF25, -11.2%) than in those with negative skin tests (FEF50, -3.5%; FEF25, -3.5% ($P < 0.01$).

Comparisons of measured preshift ventilatory capacity data with predicted normal values (Table 3) demonstrated significant decrease of FVC, FEV1, FEF50, and FEF25 in swine farmers with positive skin tests but only for FVC in those with negative skin tests.

TABLE 1

PREVALENCE OF CHRONIC RESPIRATORY SYMPTOMS IN SWINE FARMERS WITH REGARD TO POSITIVE AND NEGATIVE SKIN TESTS AND IN CONTROL WORKERS

Group	Mean age (yrs)	Mean exposure (yrs)	Chronic cough	Chronic phlegm	Chronic bronchitis	Asthma	Dyspnea	Chest tightness	Nasal catarrh	Sinusitis
Positive skin test N = 14	34	7	7 50.0% NS	6 42.9% NS	6 42.9% NS	2 14.3% NS	0 0% NS	2 14.3% NS	0 0% NS	0 0% NS
Negative skin test N = 18	36	8	6 33.3%	5 27.8%	4 22.2%	5 27.8%	0 0%	6 33.3%	0 0%	0 0%
Swine farmers N = 32	35	8	13 40.6% NS	11 34.5% NS	10 31.3% NS	7 21.9% NS	0 0% NS	8 25.0% <0.01	0 0% NS	0 0% NS
Controls N = 39	31	8	7 17.9%	6 15.4%	6 15.4%	2 5.9%	0 0%	0 0%	0 0%	0 0%

Note. NS, statistical difference not significant ($P > 0.05$).

TABLE 2

PREVALENCE OF ACUTE SYMPTOMS IN SWINE FARMERS DURING WORK SHIFT WITH REGARD TO POSITIVE AND NEGATIVE SKIN TESTS

Group	N	Cough	Dyspnea	Throat		Eye burning	Nose			Headache
				Burning	Dryness		Secretion	Dryness	Bleeding	
Positive skin test	14	12 85.7% NS	9 64.3% NS	8 57.1% NS	12 85.7% <0.05	8 57.1% NS	4 28.5% NS	4 28.5% NS	0 0% NS	3 21.4% NS
Negative skin test	18	12 66.7%	12 66.7%	7 38.9%	9 50.0%	7 38.9%	4 22.2%	5 27.8%	0 0%	5 27.8%

Note. NS, statistical difference not significant ($P > 0.05$).

TABLE 3

VENTILATORY CAPACITY IN SWINE FARMERS WITH REGARD TO POSITIVE AND NEGATIVE SKIN TEST

Group	FVC Before shift L	FVC Difference before–after shift %	FVC Difference before–after shift P	FEV1 Before shift L	FEV1 Difference before–after shift %	FEV1 Difference before–after shift P	FEF50 Before shift L/s	FEF50 Difference before–after shift %	FEF50 Difference before–after shift P	FEF25 Before shift L/s	FEF25 Difference before–after shift %	FEF25 Difference before–after shift P
Positive skin test $N = 14$	4.47 ±0.97 <0.05 5.58* ±1.04	−4.0	<0.05	3.60 ±0.67 <0.01 4.34* ±0.87	−4.4	<0.01	5.20 ±1.15 <0.05 5.65* ±0.73	−10.6	<0.01	2.50 ±0.75 <0.05 2.91* ±0.62	−11.2	<0.05
Negative skin test $N = 18$	3.60 ±0.47 <0.01 4.37* ±0.66	−3.1	NS	3.26 ±0.40 NS 3.36* ±0.53	−3.7	<0.05	5.53 ±1.18 NS 4.96* ±0.54	−3.5	<0.01	2.84 ±0.50 NS 2.69* ±0.52	−3.5	<0.01

Note. NS, statistical difference not significant ($P > 0.05$). Data are presented as mean ± SD.
* Predicted normal values.

2. Swine Confinement Building Antigen Assay in Vitro

The mean data for the effect of swine confinement building antigen (swine confinement agents) on guinea pig-isolated tracheal smooth muscle are shown in Fig. 2. A total of 17 guinea pigs were studied. The results are presented as a percentage of the initial maximal carbachol contraction produced by stimulation with 10^{-5} M carbachol.

Swine confinement building allergen increased the tone of tracheal rings inducing a dose-related smooth muscle contraction. The mean smallest maximal contractile activity was 6% (10 μl) and the mean greatest 49% (1000 μl) of the maximal carbachol contraction. Carbachol 10^{-5}M added into the organ bath at the end of the assay following swine confinement building allergen caused smooth muscle constriction similar to the initial carbachol 10^{-5}M response. A control Krebs solution added in the same volume into the organ bath did not cause changes in the tone of the guinea pig tracheal rings.

Protein determination in swine confinement building antigen demonstrated that the extract used for the in vitro study contained 252 μg/ml of protein.

DISCUSSION

Our data demonstrate that exposure of farmers in swine confinement buildings is associated with frequent respiratory symptoms, ventilatory capacity changes, and positive skin tests to swine-derived antigens. Nonexposed controls, however, also exhibited a high prevalence of positive skin tests. Differences in symptoms by skin test were not significant. By contrast the current study indicates that respiratory function changes, both acute (across-shift) and chronic (preshift), particularly those involving flow rates at low lung volumes, are more pronounced in workers with positive skin tests than in those workers with negative skin tests.
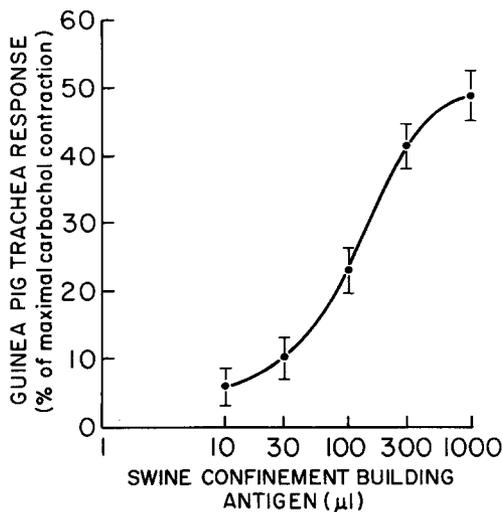

FIG. 2. Contractile activity of swine confinement building antigen on isolated guinea pig tracheal smooth muscle as percentage of initial carbachol contraction of 10^{-5}M.

Previous studies have shown no evidence that antigen–antibody reactions are important in the pathogenesis of lung disease in swine farmers. It appears that neither IgE nor IgG humoral mechanisms mediated respiratory symptoms in hog barn workers. Matson *et al.* (1983) stated that in one person with elevated IgE antibody to hog pelt, hog urine, and hog serum skin tests with these materials were negative. However, they found seven subjects with elevated IgG to hog barn dust and/or hog feed. Recently Brouwer *et al.* (1990) described an elevation of serum IgG level among pig farmers and indicated that immunological response to pig-derived antigens is related to the actual dust exposure in the confinement areas.

In our study a large number of swine farmers demonstrated positive skin tests to swine feed, swine hair, and swine confinement agents. We also found three swine workers with elevated serum IgE level and all three of them reacted to at least one of the swine skin test allergens. The presence of similar frequencies of positive tests in control workers speaks against a specific sensitization. In general, there were no significant differences seen between skin test positive and negative workers for acute and chronic respiratory symptoms, although some trends were noted. Nevertheless, lung function studies indicated that both acute across-shift changes in function as well as baseline lung function were lower in skin test positive workers. This latter finding suggests that skin reactivity to swine antigen may be a nonspecific index of airway reactivity to swine antigen.

Among our swine farmers we found a high prevalence of acute and chronic respiratory symptoms. Wilhelmsson *et al.* (1989) suggested that bacterial endotoxins and organic dust in swine confinement buildings may pose a risk for airway disease such as chronic bronchitis. A large number of our farmers complained of acute symptoms directly associated with work that were similar to the symptoms recorded by Donham (1990). A high prevalence of headache (21.4 to 27.8%) in our swine farmers may be due to exposure to different gases such as ammonia, hydrogen sulfide, carbon monoxide, or feed dust (containing components of both plant and animal origin) since this complaint was also reported by workers exposed to different types of organic dust such as animal food (Zuskin *et al.*, 1989), soy (Zuskin *et al.*, 1988c), and spices (Zuskin *et al.*, 1988a).

Swine confinement work includes exposure to different agricultural dusts since animal food consists mainly of wheat, corn, soy, sunflower, and fish flour. It may well be that positive skin tests to animal food are partly related to soy or fish flour, which are known to elicit high frequencies of positive reactions (Zuskin *et al.*, 1988c, 1989). Brouwer *et al.* (1986) found IgE antibody against soy in their swine workers. Our swine farmers with positive skin tests did not have a significantly higher prevalence of acute or chronic respiratory symptoms than those farmers with negative skin tests. However, the swine farmers with positive skin tests demonstrated significantly larger across-shift reductions and significantly decreased ventilatory capacity in relation to predicted normal values. Iversen *et al.* (1990) reported a significantly lower PC20 in symptomatic pig farmers as determined by histamine challenge. Rylander *et al.* (1990) also reported increased bronchial reactivity in pig and dairy farmers.

Our study on isolated nonsensitized guinea pig trachea suggests that clinical

respiratory findings seen in humans can be simulated in guinea pig trachea smooth muscle *in vitro*. The contractile activity is concentration dependent. We have previously shown that extract of different spices and soy dust induce airway constriction in *in vitro* experiments by a direct action on guinea pig airway smooth muscle (Zuskin *et al.*, 1988b; Schachter *et al.*, 1988). The guinea pigs used in our present *in vitro* experiments were not presensitized to swine-derived antigen. These findings imply that swine-derived antigen is capable of causing a nonimmunological response in airway smooth muscle.

This study of swine workers suggests that while positive skin tests are common in workers in this industry they are not strongly related to respiratory symptoms. Nonspecific sensitivity to these extracts may nonetheless be predictive of airway disease because of their relation to lung function abnormalities. We suggest that positive skin test responses to swine antigen may be non-IgE related and possibly reflect nonspecific reactivity. Clinical application of this finding may serve to identify workers at risk for airway effects in swine confinement areas and may help to better understand the pathogenesis of respiratory disorders in this industry.

ACKNOWLEDGMENTS

REFERENCES

British Medical Research Council Committee on the Aetiology of Chronic Bronchitis. (1960). Standardized questionnaire on respiratory symptoms. *Br. Med. J.* 2, 1665.

Brouwer, R., Biersteker, K., Bongers, P., and Houthuija, D. (1986). Respiratory symptoms, lung function, and IgG4 levels against pig antigens in a sample of Dutch pig farmers. *Am. J. Ind. Med.* 10, 283–285.

Brouwer, R., Heederik, D., and Van Swieten, P. (1990). IgG4 antibodies against pig-derived antigens. *Am. J. Ind. Med.* 17, 96–98.

Castellan, R. M., Olenchock, S. A., Hankinson, J. G., Millner, P. D., Cocke, J. B., Bragg, C. K., Perkins, H. H., and Jacobs, R. R. (1984). Acute bronchoconstriction induced by cotton dust: Dose-related responses to endotoxin and other dust factors. *Ann. Intern. Med.* 101, 157–163.

Donham, K. J. (1990). Health effects from work in swine confinement buildings. *Am. J. Ind. Med.* 17, 17–25.

DoPico, G. A. (1986). Report on disease. *Am. J. Ind. Med.* 10, 261–265.

Harries, M. G., and Cromwell, O. (1982). Occupational asthma caused by allergy to pig's urine. *Br. Med. J.* 284, 867.

Iversen, M., Pedersen, B., and Dahl, R. (1990). Relationship between respiratory symptoms and bronchial hyperreactivity in pig farmers. *Am. J. Ind. Med.* 17, 64–65.

Katila, M. L., Mantyjarvi, R. A., and Ojanen, T. H. (1981). Sensitisation against environmental antigens and respiratory symptoms in swine workers. *Br. J. Ind. Med.* 38, 334–338.

Lowry, O. H., Rosenbroug, N. J., Farra, A. L., and Randal, R. L. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–275.

Matson, S. C., Swanson, M. C., Reed, C. E., and Yunginger, J. W. (1983). IgE and IgG immune mechanisms do not mediate occupation-related respiratory or systemic symptoms in hog farmers. *J. Allergy Clin. Immunol.* 72, 299–304.

Quanjer (1983). Standardized lung function testing. Report of the working party "Standardization of

lung function tests" of the European Community for Coal and Steel, Luxembourg. *Bull. Eur. Physiopathol. Respir.* **19,** 1–95.

Rylander, R., Essle, N., and Donham, K. J. (1990). Bronchial hyperreactivity among pig and dairy farmers. *Am. J. Ind. Med.* **17,** 66–69.

Schachter, E. N., Zuskin, E., Witek, T. J., Marom, Z., Goswami, S. K., Weitzman, G., and Maayani, S. (1988). Airway smooth muscle and the constrictor effect of soy bean shell extract. *Am. Rev. Respir. Dis.* **137,** 297.

Sheldon, J. M., Lowel, R. G., and Mathews, K. P. (1967). "A Manual of Clinical Allergy," pp 507–531. Saunders, Philadelphia/London.

Wilhelmsson, J., Bryngelsson, I. L., and Ohlson, C. G. (1989): Respiratory symptoms among Swedish swine producers. *Am. J. Ind. Med.* **15,** 311–318.

Willems, H. T., Verlinden, F., and Biersieker, K. (1984). Work and health of the Dutch farmers. *T. Soc. Gerzondheidez.* **62,** 21–27.

World Health Organization (WHO) (1986). "Early Detection of Occupational Disease," pp. 37–41. Geneva.

Zuskin, E., Skuric, Z., Kanceljak, B., Pokrajac, D., Schachter, E. N., and Witek, T. J. (1988a). Respiratory findings in spice factory workers. *Arch. Environ. Health* **43,** 335–339.

Zuskin, E., Kanceljak, B., Skuric, Z., Pokrajac, D., Schachter, E. N., Witek, T. J., and Maayani, S. (1988b). Immunological and respiratory findings in spice-factory workers. *Environ. Res.* **47,** 95–108.

Zuskin, E., Mataija, M., Pokrajac, D., Schachter, E. N., and Witek, T. J. (1989). Respiratory function in animal food processing workers. *Am. J. Ind. Med.* **16,** 179–187.

Zuskin, E., Skuric, Z., Kanceljak, B., Pokrajac, D., Schachter, E. N., and Witek, T. J. (1988c). Respiratory symptoms and ventilatory capacity in soy bean workers. *Am. J. Ind. Med.* **14,** 157–165.