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Acute exposure to swine dust causes bronchial hyperresponsiveness in healthy subjects

P. Malmberg, K. Larsson

Acute exposure to swine dust causes bronchial hyperresponsiveness in healthy subjects. P. Malmberg, K. Larsson. ©ERS Journals Ltd 1993.

ABSTRACT: Six urban subjects, with little or no previous experience of farm-work, were exposed to dust while weighing swine for 2–5 h. Three subjects experienced toxic symptoms 4–5 h after the beginning of exposure. Bronchial responsiveness increased in all subjects within 6 h (more than three doubling steps difference in a methacholine test). One week later, airway responsiveness had partly normalized. The mean (interquartile range) cumulative dose of methacholine causing a 20% decrease in forced expiratory volume in one second (FEV_1) was 3.1 (1.0–6.6) mg, before exposure, fell to 0.13 (0.01–0.76) mg 6 h after exposure ($p<0.02$), and was 0.99 (0.42–1.5) mg one week later ($n=5$, $p<0.05$), Mean (SD) FEV_1 decreased 5 (2)%. The concentration of total dust varied between 9 and 14 mg·m^{-3} and of endotoxin between 0.1 and 0.5 μ g·m^{-3}.

Thus inhalation of swine farm dust, caused a marked increase in bronchial responsiveness in non-sensitized subjects.

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National Institute of Occupational Health, Solna, Sweden.

Correspondence: P. Malmberg
Respiratory Division, IFL
National Institute of Occupational Health
S-171 84 Solna, Sweden

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Respiratory symptoms, such as chronic cough, wheezing, chest tightness and fever reactions (organic dust toxic syndrome [1]), are often reported from swine farmers [2–5]. The airborne dust contains particles from feed and swine dander, micro-organisms and fragments of micro-organisms from faeces and feed [6–8]. In addition, the air is polluted with gases, such as ammonia and hydrogen sulphide from swine manure. Little or no change in respiratory function [5, 9–13], and bronchial responsiveness [13–15], has been reported in swine farmers with moderate exposure to swine dust; however, swine farmers with high exposure to swine dust appear to have increased airway responsiveness [16]. Airways inflammation, mainly characterized by increases in neutrophilic granulocytes, has been demonstrated by means of bronchoalveolar lavage [13, 17]. The agents causing the respiratory symptoms in swine farmers are not known, but endotoxin has been suggested as a contributory factor [2, 18].

The aim of the present study was to investigate whether exposure to swine dust causes changes in bronchial responsiveness and lung function. To avoid effects of sensitization and tachyphylaxis, subjects with little or no exposure to swine dust have been studied.

Subjects and procedure

Six healthy, nonsmoking, urban subjects participated in the study. One had made a brief visit to a swine confinement building more than a year earlier, the other five had no previous exposure to swine, or to other farm dusts. One had a history of urticaria (table 1, subject 1) and positive Phadiatope® test (Pharmacia, Uppsala, Sweden), the remaining five subjects had no history of atopic symptoms and were negative in the Phadiatope® assay. None had a history of asthma, and all denied respiratory infectious disease for at least six weeks prior to the study. All had normal chest radiographs. Further details are given in table 1. All participants gave their informed consent, and the study had the approval of the local Ethics Committee.

Two weeks prior to exposure to swine dust, lung function was measured, and a bronchial methacholine challenge was performed.

Table 1. – Subject characteristics and pre-exposure lung function

Subject no/sex	Age yrs	FEV_1 *l* (% pred)	VC *l* (% pred)
1/M	50	4.7(107)	6.8(116)
2/F	42	2.8(91)	3.9(101)
3/F	25	3.9(104)	4.5(98)
4/F	24	3.4(90)	4.2(90)
5/F	25	3.7(101)	4.4(103)
6/F	26	3.5(92)	4.6(100)

*: percentage predicted value according to HEDENSTRÖM [19, 20]. FEV_1: forced expiratory volume in one second; VC: vital capacity.

Table 2. – Inhalation of swine confinement building dust; exposure, changes in lung function and symptoms

Subject	Exposure	Endotoxin*	Total	Change in		$PD_{20}FEV_1$ mg			Symptoms
no	duration h	μ g·m^{-3}	dust mg·m^{-3}	PEF %	FEV_1 %	before	6 h later	1 week later	
1	5.2	0.21	6.9	-11	-4	6.6	0.01	1.4	F+M
2	4.5	0.21	6.9	-6	-4	0.3	0.00**	-	
3	2.7	0.42	14.0	-8	-3	8.5	0.89	2.1	H+M
4	2.7	0.42	14.0	-12	-4	4.9	0.76	1.0	M
5	2.3	0.40	9.9	-	-7	1.3	0.17	0.4	
6	2.3	0.40	9.9	-	-8	1.0	0.09	0.4	

*: time weighted average values; **: not measureable due to 40% fall in FEV_1 following inhalation of diluent.F: fever >39°C; M: severe malaise, necessary to lie down; H: severe headache; PEF: peak expiratory flow; FEV_1: forced expiratory volume in one second; $PD_{20}FEV_1$: provocation dose producing a 20% fall in FEV_1.

Exposure took place in a swine confinement building with approximately 700 pigs, weighing 80–110 kg. The exposure started at 9.00 a.m., and the total exposure time varied between 2–5 h (table 2). Most of the time, the subjects were moving the pigs through a weighing box.

All exposures took place in the same building, on three different occasions, with two subjects being exposed on each occasion. One subject in each pair was carrying personal samplers, collecting airborne dust for measurement of total dust and endotoxin, and exposure was assumed to be the same for both subjects in the pair. Carbon dioxide, ammonia and hydrogen sulphide were measured at mid-exposure time.

Peak expiratory flow was measured immediately before, during, and after the end of exposure, at 2 h intervals for 8 h (n=4). Lung function and bronchial responsiveness were measured on average 6 h (range 4–9 h) after mid-exposure time. Bronchial responsiveness was measured on a third occasion one week after the exposure, in five of the subjects. On the day of exposure, each subject measured oral temperature at about 2 h intervals. Venous blood samples were taken immediately before exposure, and before measurements of lung function 4–9 h after mid-exposure time.

Methods

Bronchial responsiveness was measured by a methacholine provocation test. Inhalation of diluent was followed by doubling doses of methacholine, starting at 0.5 mg·ml^{-1}, until forced expiratory volume in one second (FEV_1) had decreased 20% compared to the value obtained after inhalation of diluent. The cumulative dose causing a 20% decrease in FEV_1 ($PD_{20}FEV_1$) was calculated [21]. The method was standardized, with control of inhalation flow (0.4 *l*·s^{-1}), inhalation volume (0.8 *l*), and number of breaths. The output of the nebulizer (0.38±0.01 ml·min^{-1}) was measured daily. The details of the procedure have been described previously [21].

The FEV_1 (best of three blows), and vital capacity (VC) (best of three slow and three forced vital capacities), were measured with a low-resistance rolling-seal spirometer (OHIO model 840, Airco, Madison, Wisconsin, USA). Local reference values were used [19, 20]. Peak expiratory flow (PEF) was measured with a mini-Wright® peak flow meter (Clement Clarke Ltd, London, UK), and the best of three blows was chosen. The leucocyte count in venous blood was measured with a Coulter® STKD counter (Coulter electronics Ltd, Luton, UK).

Total dust and endotoxin was sampled with 25 mm open-phase filter cassettes, at an air flow of two *l*·min^{-1}, for the full duration of exposure. The cassettes were changed at intervals, and a total of nine samples from the three exposure situations was obtained. The cassettes were equipped with cellulose acetate filters (Millipore® AAWP filters, Cork, Ireland), and were carried in the breathing zone. Total dust was measured by weighing after 24 h of conditioning, using a Mettler® ME 22 balance (Mettler, Greisensee, Switzerland) and reference filters. Endotoxin was extracted from the filters by shaking 10 ml of pyrogen-free water in a Stomacher washer. Endotoxin was measured after suitable dilution with a chromogen version of the Limulus amoebocyte lysate assay [22, 23] (CoA endotoxin test, Kabi Vitrum diagnostica, Stockholm, Sweden, with *Escherichia coli* 0111:B4 as standard). The results of endotoxin and total dust measurements were expressed as time weighted average concentration for the duration of the exposures. Carbon dioxide, ammonia and hydrogen sulphide were measured with a multi-gas detector (Dräger AG model 21/31, Lybeck, Germany).

The "biological" dose of inhaled endotoxin was calculated, assuming 50% retention, a ventilation of 1.5 m^3·h^{-1} and underestimation of the biologically effective endotoxin dose in air samples by a factor of three [24]. Wilcoxon's signed rank test was used for statistical comparisons.

Results

Three subjects experienced malaise, drowsiness and a fainting sensation, 4–6 h after the beginning of exposure. One also had severe headache, 5 h after the start of exposure. In one subject (table 2, subject 1), body temperature was stable for 6 h, rose to a maximum of 39.1°C, 9 h after the start of exposure, and was normalized the next morning. The other subjects had temperature changes of less than 1°C. The mean (SD) venous

blood leucocyte count increased from 5.6 (1.9) cells·10^9 before exposure to 11.2 (3.6) cells·10^9 6 h after exposure.

All subjects had a more than sixfold decrease in $PD_{20}FEV_1$ recorded 6 h after mid-exposure time, compared to pre-exposure values (table 2). The median (25–75th percentile) $PD_{20}FEV_1$ decreased from 3.1 (1.0–6.6) mg pre-exposure to 0.13 (0.01–0.76) mg 6 h after exposure ($p<0.02$) (table 2). In one subject, inhalation of diluent caused a 40% decrease in FEV_1, 6 h after exposure to swine dust (table 2). One week after exposure, $PD_{20}FEV_1$ had increased slightly to 0.99 (0.42–1.5) mg ($p<0.05$, n=5), but was still lower than pre-exposure values ($p<0.05$).

FEV_1 was on average 5 (2)% lower 6 h after mid-exposure time, compared to the pre-exposure value ($p<0.01$) (table 2). PEF decreased between 6-12%, with minimum values 2–4 h after the start of exposure (n=4). VC was not significantly altered.

The duration of exposure and the concentration of total dust and endotoxin in air are given in table 2. The estimated "biological dose" of inhaled endotoxin was 2.3 (0.3) μ g. The concentration of ammonia in air varied between 2 and 3 parts (per million) (ppm), carbon dioxide between 0.08 and 0.1 volume-percent, and hydrogen sulphide was less than 0.05 ppm on all occasions.

Discussion

The most conspicuous finding in the present study was the marked increase in bronchial responsiveness by more than 3 doubling doses, which occurred in all subjects within hours after exposure to dust in a swine confinement building. The 95% confidence interval for duplicate measurements of bronchial responsiveness in healthy subjects, using the same equipment as in the present study, is about 2–2.4 doubling doses [21]. The method of measurement of bronchial responsiveness was carefully standardized and the measurements were performed by an experienced investigator. The output of the nebulizer was checked daily, before and after provocations, and the measurements were performed on different weeks for the three pairs of exposed subjects. FEV_1 measured 6 h after the beginning of exposure was only 5% below pre-exposure values. It is, therefore, unlikely that the increase in bronchial responsiveness was caused by geometrical effects of bronchoconstriction [25]. Bronchial responsiveness was partly normalized, but still increased significantly, one week after exposure.

It is unlikely that immunological sensitization to swine dust contributed to the change in brochial responsiveness, since the subjects in the present study had little or no previous exposure to swine confinement building dust. Previous studies in Swedish swine farmers have indicated normal [13], or slightly increased [15], airway reactivity. However, heavily exposed swine workers appear to have increased airway responsiveness [16]. Most studies on work shift changes in FEV_1 in swine farmers have shown little or no change [2]. The difference in reaction to swine farm dust between previously non-exposed subjects and swine farmers indicates that the latter group may have attenuated responses due to tachyphylaxis. This is similar to tachyphylaxis in "mill-fever" in the cotton industry (symptoms are worse on the first workday after a prolonged leave) [26]. Animal experiments have demonstrated decreased neutrophil accumulation following repeated exposure to endotoxin [27, 28], which has been suggested as a possible causative agent of respiratory symptoms in swine confinement workers [18].

The experimental protocol was designed to maximise exposure associated with symptoms. The procedure of weighing fattened swine is performed about once a week for about 5 weeks, three times a year, in a large unit. Weighing of swine requires a longer stay in the barn than normal, and is associated with febrile reactions [29]. Three subjects in the present study had symptoms of "organic dust toxic syndrome" [1]. Thus, absence of symptoms during every-day farming could be explained by the neccessity of achieving a threshold value of exposure before symptoms occur.

Several agents in the dust may cause bronchial inflammation, which could relate to the pathogenesis of bronchial hyperresponsiveness. Grain sorghum dust extracts have pronounced inflammatory effects when given intratracheally to experimental animals, and endotoxin- depleted grain dust activates complement and has neutrophil chemotactic activity [30]. Pig-derived material, such as dander and faeces, may also have contributed. Particulate organic material may activate complement (review [31]). The total number of particles of respirable size are in the order of 10^7–$10^8 \cdot m^{-3}$ (electron microscopic data from swine farms, not reported), and many of these have a flat shape [8], which helps them to remain suspended in the air. Crushed barley is the dominant source of these respirable particles [8]. Swedish barley contains about 4% mixed β-(1-3), (1-4)-D-glucan [32], and at least 1% of the glucans are in the form of long chains of β-(1-3)-D-glucan [33], which activates alveolar macrophages *via* interaction with a specific receptor [34].

Inhalation of endotoxin from the cell wall of Gram-negative bacteria causes a transient rise in bronchial responsiveness in rats [35]. In human volunteers, 300 μ g inhaled "biological dose" of endotoxin (30 μ g according to Limulus-assay) in the form of Gram-negative bacteria caused no change in $PD_{15}FEV_1$ [24]. However, provocation with 4 mg of methacholine resulted in a significantly larger FEV_1 decrease 4 h after inhalation of endotoxin, compared to measurements made before exposure. There was no significant difference 24 h after exposure to endotoxin. This is at variance with the present study where bronchial responsiveness ($PD_{20}FEV_1$) was decreased sixfold 6 h post exposure and was not fully normalized one week later.

Endotoxin concentration in air in a total of 228 swine confinement buildings typically varied between 0.1-0.2 μ $g \cdot m^{-3}$ [10, 11, 13, 36, 37]. These values relate to every-day activities rather than to infrequent activities such as weighing of swine before slaughter. Higher values have been reported during feeding than during tending of swine [13]. In the present study, the endotoxin concentration varied between 0.1–0.5 μ $g \cdot m^{-3}$ in nine samples.

The estimated average "biological dose" of endotoxin

was 2.1 μ g in the present study. This value is low compared to the 300 μ g of cell-bound endotoxin, which was used in the inhalation study mentioned above [24], causing a much smaller change in bronchial responsiveness. The present study, therefore, does not support the hypothesis that endotoxin alone is responsible for most of the change in bronchial responsiveness caused by inhaled swine dust. It cannot be ruled out that hyperresponsiveness could have been caused by endotoxin in conjunction with a co-factor influencing airway deposition or activity. Perhaps a more likely explanation is that other agents enhancing airway inflammation and release of inflammatory mediators are involved in the pathogenesis. Increased concentrations of neutrophils [13, 17] and albumin, fibronectin and hyaluronan have been demonstrated in the bronchoalveolar lavage fluid of swine farmers [13] suggesting inflammation. The causes of the inflammation are not known, but they may be related to the deposition in the lower airways of large numbers of particles containing β-glucans and attached endotoxin. Such particles could, among other effects, cause complement activation and cytokine release *via* interaction with specific receptors [8, 31, 33].

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