

Effects of repeated swine building exposures on normal naive subjects

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ABSTRACT: Exposure to swine confinement buildings has a negative impact on respiratory health. A short exposure to this environment results in an acute airway inflammatory response. The present study was performed to confirm and further define the acute effects of working in a swine building, and to determine whether these effects are reproducible.

Seven previously nonexposed normal subjects underwent evaluations that included hourly measurement of forced expiratory volume in one second (FEV₁), methacholine challenge (the provocative concentration producing a 20% fall in FEV₁ (PC₂₀)), bronchoalveolar lavage (BAL), nasal lavage (NL), and blood analyses, before (control) and after each of two 5 h exposures to a swine building environment. The exposures were conducted 8 days apart. The levels of total dust, endotoxins, and ammonia (NH₃) in the confinement building were measured on each day of exposure.

Both exposures resulted in a significant reduction in FEV₁ (mean±SEM change in FEV₁: control = 7±2%; exposure 1 = 15±3%; exposure 2 = 23±3%), decrease in PC₂₀ (median value (25th-75th percentile): 223 (23-256), 20 (15-198) and 20 (11-71), respectively; p=0.05) and increase in BAL cells (129±20, 451±43 and 511±103×10³ cells·mL⁻¹, respectively) and NL cells (6±4, 126±58 and 103±26×10³ cells·mL⁻¹, respectively), mostly neutrophils. Levels of interleukin-8 (IL-8), but not interleukin-1 (IL-1) or tumour necrosis factor-α (TNF-α), increased both in BAL and nasal fluids with exposure.

In normal naive subjects, repeated exposure to the environment of a swine building induced a marked and reproducible reduction in forced expiratory volume in one second, increase in airway responsiveness, and increased neutrophilic inflammatory response. These results could not be accounted for by any of the environmental factors measured.

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Confining large numbers of animals in relatively small spaces has become the standard practice of swine production in most industrialized countries. Studies have documented that the air in swine confinement buildings contains large quantities of dust, bacteria and fungi, endotoxins and toxic gases (hydrogen sulphide (H₂S) and ammonia (NH₃) being the most abundant) [1-5]. In cold countries, such as Canada, adequate ventilation required to assure proper elimination of these contaminants is economically unfeasible, particularly during the winter months, when ventilation is reduced to conserve heat inside the swine building.

Exposure to swine buildings is a respiratory health hazard [6-12]. Farmers who work in these buildings have a high prevalence of chronic bronchitis [13-15], increased airway responsiveness [16-18], and a decline in their lung functions during the shift [19, 20]. Separate Swedish studies [21, 22] showed lung inflammation and airway hyperresponsiveness after a brief exposure of naive subjects to swine building dust. No information is provided on the possible link between these two important observations. These acute results are surprising, since chronically exposed pig farmers have only a very mild

increase in bronchoalveolar lavage (BAL) cells and no increase in airways reactivity to histamine [23, 24]. This discrepancy could be explained either by an adaptation process in farmers or by a selection process, with bias towards those who can tolerate the polluted environment.

We designed this study: 1) to determine whether swine building exposure in Quebec induced similar effects in normal naive subjects as weighing pigs did in Sweden; 2) to determine whether the effects of swine building exposure were reproducible; 3) to measure early phase cytokine involvement in inflammation due to swine building exposure; 4) to evaluate less invasive parameters than BAL, e.g. nasal lavage, white blood cells (WBCs), to monitor the effects of this exposure; and 5) to look at environmental contaminants that could induce changes in the parameters measured. The study of air contaminants simultaneously with subject exposure could help identify potential risk factors that need to be controlled. The study of reproducibility is important for future studies in which environmental interventions would be tested. Any parameter that is reproducible, especially if noninvasive, could serve as a marker of the efficacy of interventions to improve air quality.

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Materials and methods

Subjects

Seven nonsmoking healthy males, aged 24–38 yrs (mean 29 yrs), who had never been exposed to the environment of a swine confinement building participated in the study. Each subject was first screened by spirometry and skin-prick tests to common aeroallergens, including pig antigens. Subjects normal in these aspects, with no history of lung disease or recent airway infections (<1 month), and on no medication were eligible for participation. Eleven young males were screened to identify the seven eligible subjects. Four were eliminated, one due to a recent history of smoking and three because of positive skin-prick reactions to common airborne allergens. Before starting participation, each subject signed a consent form approved by our institution's Ethics Committee.

Swine confinement building

The building was selected to represent an average type in Quebec. It housed about 400 swines, aged 2–5 months, *i.e.* the fattening period before market. The building was ventilated by eight temperature-controlled fans placed on each side of the building. During the winter months, the period when the experiments were performed, the inside temperature was set at 16°C.

Study design

Day 1. Nonexposure baseline day with hourly measurements of forced expiratory volume in one second (FEV₁), body temperature, and symptom scores (scale 1–10) for headache, dyspnoea, cough, chest tightness, chills, running nose, and eye irritation, from 09.00 to 14.00 h. Venous blood was collected and nasal lavage obtained at 16.00 h, for cell counts and cytokine determinations.

Day 2. Methacholine challenge (starting dose of 8 mg·mL⁻¹) and BAL (between 07.00 and 09.00 h).

Day 10. Swine building exposure from 09.00 to 14.00 h with measurements and assessments of the same parameters as on Day 1, including venous blood sampling and nasal lavage at 16.00 h.

Day 11. Methacholine challenge (as for Day 2 but with a starting dose of 2 mg·mL⁻¹) and BAL (between 07.00 and 09.00 h).

Day 18. Repeat of Day 10.

Day 19. Repeat of Day 11.

On both exposure days air, samples were collected for the measurement of total dust, endotoxin and NH₃ levels in the swine building. An experienced nurse, trained in performing spirometry, accompanied all volunteers to the swine building to ensure their safety and that the study parameters were accurately followed.

Spirometric measurements

Forced expiratory flows and volumes were obtained using a Vitalograph spirometer (Roxon, Buckingham, UK). On the initial evaluation, we obtained complete

flow curves from which the forced expired vital capacity (FVC), FEV₁ and the ratio FEV₁/FVC were derived. The tests were performed according to the American Thoracic Society (ATS) standard procedure [25]. For the methacholine challenges and hourly monitoring (see protocol) only FEV₁ measurements were obtained.

Methacholine challenge

A standard procedure as described by JUNPER *et al.* [26] was used in this study. Briefly, doubling doses of methacholine were delivered *via* a calibrated Wright nebulizer, and inhaled for 2 min every 5 min until there was a drop in FEV₁ of at least 20% or a maximal dose of 256 mg·mL⁻¹ had been reached. The provocation concentration giving a 20% fall in FEV₁ (PC₂₀) was obtained by interpolation on a semilogarithmic scale.

Nasal lavage

After blowing and wiping his nose, the subject was instructed to occlude his retropharynx by positioning the tongue against the soft palate. He then tilted his head backward, and 5 mL of 0.9% saline was instilled into each nostril. The saline wash was kept in the nasal cavities for 20 s, while the subject held his breath and maintained a closed posterio-pharynx and a backward tilted head position. The subject then tilted his head forward and blew the nasal wash into a clean dry flask [27]. The volume of fluid recovered was measured, before centrifuging at 500×g for 15 min at 4°C. The supernatant was aliquoted and frozen at -70°C for subsequent cytokine assays. The cell pellet was resuspended in 100–500 µL of Hank's balanced saline solution (HBSS) for total cell count. Differential counts were performed on Diff-Quik stained glass coverslip slides [28]. Cell viability was verified by trypan blue exclusion.

Bronchoalveolar lavage

Under local anaesthesia, a 5.5 mm outer diameter fiberoptic bronchoscope was advanced and wedged into a segmental bronchus. The wedged lung segment was then lavaged with five aliquots of 60 mL of 0.9% saline; the fluid was gently aspirated after each aliquot. The fluid recovered was processed, as described for the nasal lavage, to obtain total cell and differential counts. BAL fluids were concentrated 15 fold by centrifugation using a 10,000 cut-off centricon filter (Amicon Canada Ltd), and aliquots were frozen at -70°C for cytokine measurement.

Blood samples

Ten millilitres of heparinized venous blood were withdrawn. Total and differential counts were obtained electronically with a cell counter (STKS; Coulter Electronics Ltd, Hialeah, FL, USA). Another 10 mL of non-heparinized blood was withdrawn, allowed to clot, and the serum separated by centrifugation. Serum was kept frozen at -70°C until processed for cytokine determination.

Cytokine measurements

Levels of tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-8 (IL-8) in nasal lavage

fluids, concentrated BAL fluids and sera were assayed by enzyme-linked immunosorbent assay (ELISA) kits from Perceptive Diagnostics (Cambridge, MA, USA).

Air sampling and measurements

Total dust was measured by personal sampling ($1.5 \text{ L}\cdot\text{min}^{-1}$) for the total exposure time. Dust was deposited on a dry preweighed filter cassette. The dust was dried on the filter for 24 h, the filter with dust was weighed by gravimetry (with a precision of 0.01 mg), and the average amount of environmental dust for the 5 h sampling period was calculated. Endotoxin was measured from total dust by Chromogenic Limulus Amoebocyte Assay (Associates of Cape Cod, Woodshole, MA, USA). Dust suspension in sterile endotoxin-free water (LAL reagent water) (Associates of Cape Cod) was obtained from the filter. Dilutions of the samples were made directly in a Pyroplate (Associates of Cape Cod), and endotoxins were measured using pyrochrome. The dilutions giving an optical density of 0.2–0.5 were used to calculate the amount of lipopolysaccharides (LPS) per cubic metre of air. To measure ammonia levels, air was sampled at $0.15 \text{ L}\cdot\text{min}^{-1}$ through a sulphuric acid pretreated silica gel column, and ammonia was measured by spectrophotometry.

Statistical analysis

Data were expressed graphically as mean \pm SEM. Blood cell counts were first analysed using repeated-measures analysis of variance (ANOVA), with sphericity tests on orthogonal components to test for no difference between baseline, exposure 1 and exposure 2. This test determines whether or not the F tests from univariate design are valid. Since the p-values of sphericity tests were almost significant at the 0.05 level, only repeated measures designs were used. The same approach was used for BAL and nasal wash cells and lung functions. Comparisons between baseline and exposure 1 and between baseline and exposure 2 were performed using Student's paired t-test. To ensure an overall p-value of 0.05, a Bonferroni's correction was applied to these comparisons. Thus, 0.05 was divided by 2 to test at the 0.025 level. All other tests were performed at the 0.05 level. The data were analysed using the Statistical Analysis System program (SAS Institute Inc., Cary, NC).

Results

Symptoms

Maximal scores for symptoms that occurred on either of the exposure days are presented in table 1. Most subjects reported nasal congestion, four had some cough, four a headache and two mild eye irritation. Three subjects developed mild dyspnoea, and one had a chill the evening following his second exposure. At that time, his oral temperature was 38.4°C . Otherwise, body temperature remained normal in all subjects.

Lung functions

Changes in FEV₁ for each subject are presented in figure 1. The data represent the maximal difference

Table 1. – Maximal subjective scores, on a scale of 0–5 (0=no symptoms, 5=severe symptoms), for different symptoms reported by each volunteer on either of the exposure days

Ss No.	Eye irritation	Nasal congestion	Chills	SOB	Cough	Headache
1	0.5	3	0	1	1.5	2.5
2	0	2	0	0	2	0
3	0	2	0	0	3	0
4	1	2	0	1	2	1
5	0	3	2	0	0	3
6	0	1	0	2	0	2
7	0	0	0	0	0	0

Ss: subjects; SOB: shortness of breath.

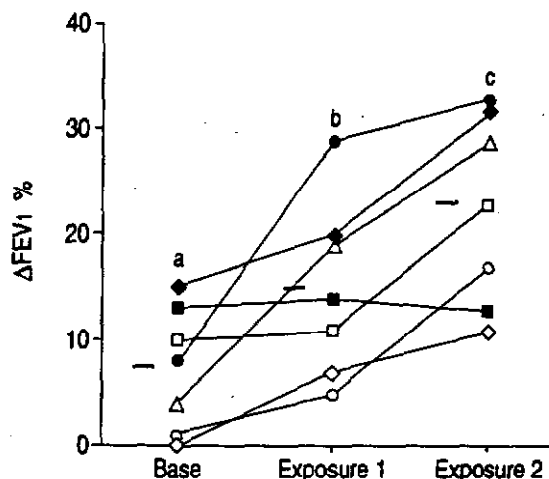


Fig. 1. – Maximal changes in forced expiratory volume in one second (ΔFEV_1) for each subject on the three study days. Horizontal lines represent the mean values. The different letters represent statistically significant differences; all p-values were <0.02 .

Table 2. – Provocation dose of methacholine ($\text{mg}\cdot\text{mL}^{-1}$) causing a 20% drop in FEV₁ (PC₂₀) for each subject, for the baseline day and after each exposure day

Ss No.	PC ₂₀ methacholine $\text{mg}\cdot\text{mL}^{-1}$		
	Base	Exposure	Exposure 2
1	14.5	4	3.4
2	190	256*	256*
3	256*	26	24
4	256*	14	11
5	256*	19.5	10.4
6	ND	256*	87
7	23	20	20
Median (25–75th percentiles)	223 (23–256)	20 (15–198)	20 (11–71)

*: the value of $256 \text{ mg}\cdot\text{mL}^{-1}$ is given when the FEV₁ did not drop by 20% at this maximal dosage. Ss: subjects; FEV₁: forced expiratory volume in one second; ND: not determined for technical reasons.

between the highest and the lowest FEV₁ obtained on each study day for each subject. The FEV₁ drop was significantly greater on both exposure days compared to the control baseline day ($p=0.02$). The second exposure day induced an even larger decrease in FEV₁ than the first ($p=0.009$). Individual values for PC₂₀ methacholine are presented in table 2. Four of the six subjects for whom we have measurements for all three study

days had a significant reduction in their PC20 (greater than a twofold concentration). In one subject the PC20 fell into the classical asthmatic zone ($<8 \text{ mg}\cdot\text{mL}^{-1}$). PC20 at baseline was higher than on the swine exposure days ($n=6$; $p=0.05$). The difference in PC20 between the two swine days was also nonsignificant ($n=7$; $p=0.33$). Subjects who had a drop in their PC20 were re-challenged 2–3 weeks later: all PC20s had returned to pre-exposure values (data not shown).

BAL, nasal lavages and blood

There were no purulent secretions in the airways at any of the bronchoscopies; however, the bronchoscopist (ML) noted mild-to-moderate airway inflammation after swine building exposures. Figure 2 presents the BAL cellular findings before and 24 h after each exposure to the swine building. The total number of cells increased significantly ($p=0.0002$). The number of macrophages doubled ($p=0.0003$), the lymphocytes increased from 21.9 to 75.8 and $117.3 \times 10^3 \text{ cells}\cdot\text{mL}^{-1}$ BAL fluid ($p=0.0006$), while the neutrophils had the greatest increase, from 2.7 to 142.2 and to $145.0 \times 10^3 \text{ cells}\cdot\text{mL}^{-1}$ ($p=0.0276$). Results of the nasal washes are presented

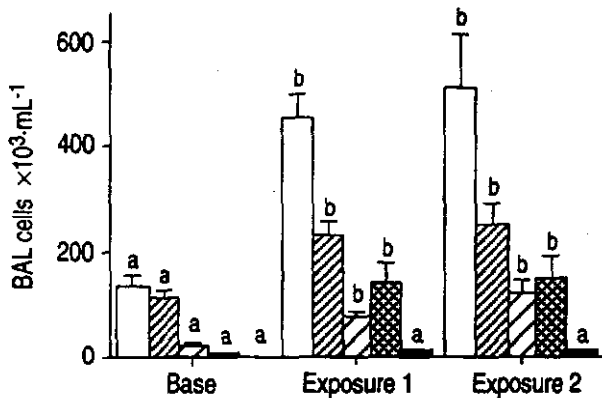


Fig. 2. — Total number and differential of bronchoalveolar lavage (BAL) cells ($\text{cells}\cdot\text{mL}^{-1}$ of BAL return). Values are presented as mean \pm SEM. For each cell type, columns with different letters are statistically different; all p -values were <0.0276 . □: total cells; ▨: macrophages; ▤: lymphocytes; ▥: neutrophils; ▧: eosinophils.

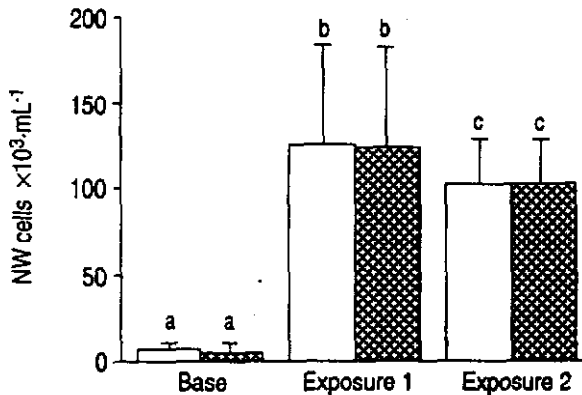


Fig. 3. — Nasal wash (NW) cellular returns and differentials ($\text{cells}\cdot\text{mL}^{-1}$ of recovered fluid for the three study days). Values are presented as mean \pm SEM. The large increase in nasal lavage cells was due mainly to the recruitment of neutrophils. Letters above each column have similar meanings as for figure 2; all p -values were <0.013 . □: total cells; ▥: neutrophils.

in figure 3. Seven hours after the start of the first exposure, there was a 10–30 fold increase in the total number of cells recovered compared to baseline values ($p=0.013$); these cells were mostly neutrophils. The increase in cell numbers was also seen after the second exposure ($p=0.010$). This response was, however, significantly lower than that seen after the first exposure ($p=0.016$). The systemic effect of swine building exposure is illustrated by the results in WBC counts (fig. 4). The increase in total WBCs was highly significant ($p=0.001$); this increase was due mainly to an increase in the number of neutrophils.

IL-8 levels in concentrated BAL fluids and in the nasal washes, before and after both exposures are presented in figure 5. This cytokine was significantly increased after exposure in both fluids ($p=0.016$ and $p=0.0001$, respectively). No IL-8 was detected in the serum. IL-1 levels in BAL fluids and nasal washes were variable and not significantly increased in the BAL fluid after the exposures ($p=0.149$ and $p=0.265$, respectively compared to baseline) (fig. 6). TNF- α concentrations in BAL fluids, nasal washes and sera, at the time-point measured, were not altered by swine building exposures ($p=0.578$, $p=0.164$, and $p=0.287$ respectively) (fig. 7).

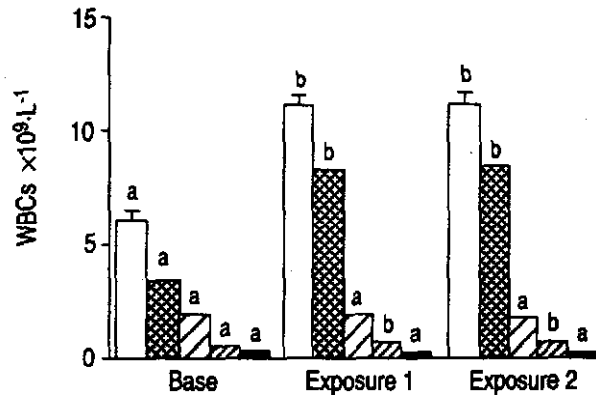


Fig. 4. — White blood cell (WBC) counts for each study day. Values are presented as mean \pm SEM. Some error bars are not visible. As for the nasal lavage, neutrophils accounted for the overall increase. Letters above each column have similar meanings as for figure 2; all p -values were <0.0274 . □: total cells; ▨: monocytes; ▤: lymphocytes; ▥: neutrophils; ▧: eosinophils.

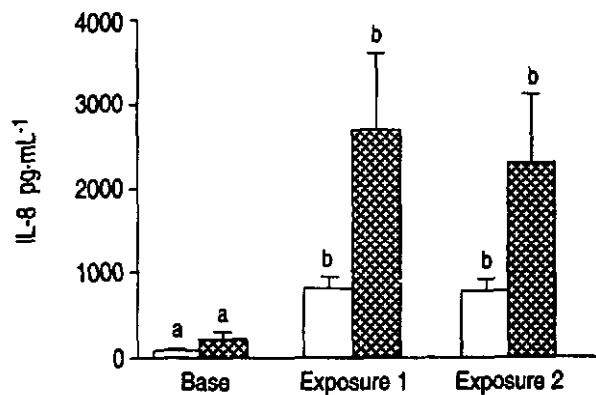


Fig. 5. — Quantity of interleukin-8 (IL-8) measured in nasal wash and bronchoalveolar lavage (BAL) fluids on each study day. Values are presented as mean \pm SEM. This cytokine was significantly increased in both fluids after each swine exposure. Letters above each column have similar meanings as for figure 2; $p=0.016$ for BAL fluid; $p=0.00001$ for nasal fluid. □: BAL fluid; ▥: nasal washings.

Environmental data

Total dust, endotoxins and NH_3 measured for each subject by personal samplers are presented in table 3. The missing data were due to technical problems. The personal sampler of subject No. 2 fell into the dung pit on his second exposure day! LPS levels were significantly higher on the first exposure day than on the second ($p=0.0001$), the other parameters were similar on

both days. The outside temperature on the seven different days that we visited the swine confinement building ranged from -3 to -22°C .

Discussion

The present study confirms that exposure to a swine building can cause a marked inflammatory response, acute symptoms, and an increase in bronchial responsiveness. This study also shows that a second exposure, 8 days later, provoked a similar type of response. No specific environmental parameter has been identified to explain all the findings.

Although the number of subjects studied is small, the results are sufficiently striking to confirm the effect of a short-term exposure to a swine building environment in previously normal nonexposed subjects. Only a small number of subjects was included due to the complexity of the study. Because of this small number of subjects no valid correlations between variables could be made.

Because of the small number of subjects studied, we felt it would be best to perform all the exposures in the same building. The fans of the building where subjects were exposed were set to function on the basis of the inside temperature, which was kept at 16°C . Ventilation was, therefore, influenced by the outside temperature. The study was performed in January and February, two cold months in Quebec, when minimal ventilation would be used. The outside temperature was below 0°C on all study days.

Our results support those of previous studies [21, 22], showing that naive subjects exposed acutely to swine confinement buildings seem to have more intense inflammatory response than workers exposed chronically to this environment [23, 24]. Moreover, chronically exposed subjects have smaller shift changes in their lung functions [20], and do not have increased bronchial reactivity [24]. These differences could represent either a selection process, where only tolerant subjects continue to work in swine buildings, or an adaptation to the environment.

Although both exposure days resulted in striking responses compared to baseline, cross-shift changes in FEV1 and nasal lavage cell numbers were significantly different between the two exposure days. The greater decrease in FEV1 on the second exposure could represent residual effects of the first exposure [21]. Whilst the

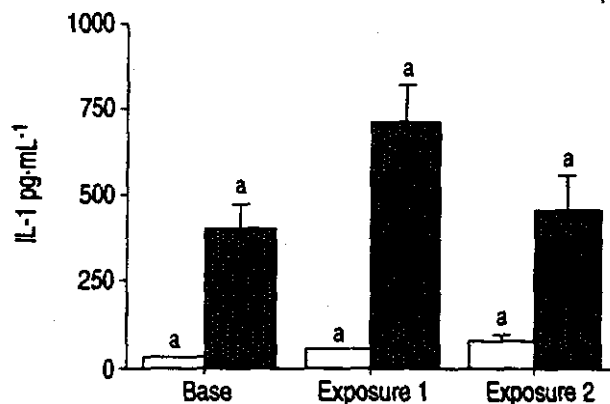


Fig. 6. - Interleukin-1 (IL-1) measured in bronchoalveolar lavage (BAL) fluid and nasal washings on each study day. Values are presented as mean \pm SEM. As shown by the same letters above each column, the differences did not reach statistical significance; $p>0.14$. □: BAL fluid; ■: nasal washings.

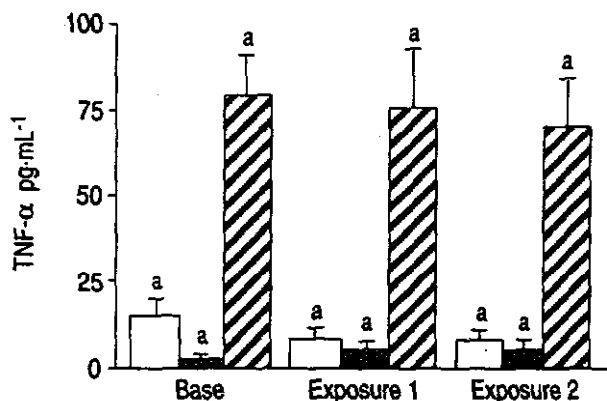


Fig. 7. - Levels of tumour necrosis factor-alpha (TNF- α) measured in lavage fluids and serum before and after each exposure to the swine building. Values are presented as mean \pm SEM. Swine building exposure had no effect on the level of this cytokine, as shown by the same letters above each column; $p>0.164$. □: BAL fluid; ■: nasal washings; ▨: serum.

Table 3. - Results of personal air sampling for each subject on each exposure day

Ss No.	Total dust $\text{mg}\cdot\text{m}^{-3}$		Ammonia $\text{mg}\cdot\text{m}^{-3}$		LPS $\mu\text{g}\cdot\text{m}^{-3}$	
	Exposure 1	Exposure 2	Exposure 1	Exposure 2	Exposure 1	Exposure 2
1	51.6	2.6	6.4	9.9	78.6	8.7
2	6.5	ND	11.0	ND	16.5	ND
3	6.7	11.9	11.9	17.7	25.6	7.9
4	18.7	8.8	21.7	6.3	14.1	7.9
5	ND	6.2	7.3	14	ND	6.4
6	8.3	12.1	2.4	18	19.6	6.9
7	9.9	9.2	10.2	9	3.7	9.8
Mean	17.0	8.5	10.1	12.4	28.0	7.9
\pm SEM	± 7.2	± 1.5	± 2.3	± 1.9	± 10.3	± 0.5
p-value	0.70		0.47		0.0001	

Ss: subjects; LPS: lipopolysaccharide; ND: not determined.

FEV₁ decreases were greater, the nasal inflammation was lower on the second day of exposure. It is unclear whether there is a relationship between the two. One could speculate that subjects breathed through the mouth more on the second exposure day, and therefore had less nasal inflammation and more bronchial response secondary to bypassing the protective filter of the nose and sinuses. Further studies comparing the route of breathing, *i.e.* nose versus mouth, on responses will be needed to verify this hypothesis. The responses between both exposures were, however, similar for all other variables, which could, therefore, be used as outcome parameters to study the efficacy of environmental interventions. These results are similar to those reported from Sweden after weighing pigs [21, 22].

The reproducibility and the magnitude of the increase in venous blood WBCs suggests that this simple outcome parameter could be useful in evaluating the effectiveness of environmental interventions on human response to swine confinement buildings. It is unclear, however, whether the factor (or factors) in the air that induced this blood response is the same as that which induced the lung reactions. Similar comments are also true for the nasal lavages.

Considering the acute and intense nature of the inflammatory response in the lungs and the peripheral blood to the swine building exposure, it is not surprising that acute phase cytokines are released in the process [29]. As reported previously [30], IL-8, which acts as a chemoattractant and activator of neutrophils at the site of inflammation, was markedly increased, and, therefore, was probably involved in the recruitment of the neutrophils at the different sites. The lack of large increases in serum levels of TNF- α after swine environment exposure corroborates the findings of MALMBERG and co-workers [31]. However, WANG and co-workers [32, 33] did find a small increase in BAL, nasal wash and serum levels of TNF- α . This discrepancy can be explained by the difference in sensitivity of the cytokine measurement kits used. The levels of this cytokine reported by WANG and co-workers [32, 33] were below the detection limits of the kit used in the current study. It seems, therefore, that TNF- α may also be involved in the response to swine confinement building exposure. This is supported by a time course study of interleukin-6 (IL-6) and TNF- α following the inhalation of swine dust [32]. Although generation of IL-1 seemed to increase, the differences were not significant. This lack of significant difference is explained by the large variations in its levels. In a previous study, WANG and co-workers [32] showed an increase in IL-6 in the serum of subjects exposed to swine confinement environments. The exact cascade of cytokine production and release and the respective role of each in the inflammatory response to swine building exposure remains to be clarified.

The current study failed to identify any single environmental factor that could explain all of the findings. Contrary to previous reports with exposure to grain dust, endotoxin levels were not sufficient to explain the present findings [34, 35]. The greater quantities of LPS on the first exposure day were not associated with a greater lung or WBC response on that day.

In conclusion, the current study confirms that short-

term exposure to a swine building induces an acute inflammatory effect in normal naive subjects. Swine buildings in Quebec appear to be similar to those in Sweden. Although there are differences in response between the two exposure days, there are sufficient similarities and reproducibility in the observations made, so that similar studies could be valid to evaluate the efficacy of environmental control measures and personal protection devices. The mechanisms and environmental factors responsible for the effects observed remain to be elucidated. Further studies are needed to verify whether exposure to all swine confinement buildings induces similar responses, and, if not, what differences between different buildings could explain why one swine building is less hazardous than another.

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