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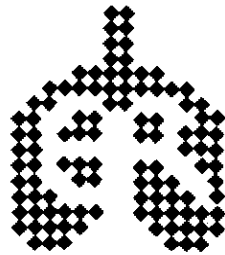
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Farming practices and the respiratory health risks of swine confinement buildings

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Farming practices and the respiratory health risks of swine confinement buildings. Y. Cormier, E. Israël-Assayag, G. Racine, C. Duchaine. ©ERS Journals Ltd 2000.
ABSTRACT: This study investigated whether clean swine confinement buildings (SCB) are less harmful to the respiratory system than older and dirtier facilities.

Eight healthy volunteers were exposed for **4 h**, at 1 week intervals, to eight SCB representing the widest possible range of cleanliness. Each volunteer and a technician rated the SCB for cleanliness from 1–10, 1 being the cleanest possible. Airborne dust, bacteria, endotoxin levels, molds, and ammonia were measured. For each volunteer measured, before and after each exposure, forced expiratory flows (forced expiratory volume in one second ($FEV1$), and forced vital capacity), white cells in nasal wash and venous blood, and nasal lavage levels of interleukin (IL)-8 and serum levels of IL-6. A methacholine challenge was obtained at baseline and following each exposure.

Cleanliness scores ranged 1.5–8.25. Mean airborne levels were: dust 3.54 $mg \cdot m^{-3}$, bacteria 4.25×10^5 $CFU \cdot m^{-3}$; endotoxins 404 $EU \cdot m^{-3}$; molds 883 $CFU \cdot m^{-3}$; ammonia 20.7 parts per million (ppm). Expiratory flows decreased after exposure ($FEV1$ from 4.8±0.7 to 4.4±0.7, $p<0.001$), neutrophils in the nasal wash and white blood cells increased (28.5±37 to 424±207 × 10^3, 5.4±1.0 to 7.4±1.7 × 10^9 cells·mL^{-1} respectively), IL-8 increased from 158±311 to 2679±639 pg·mL^{-1}, IL-6 from 0.15±0.26 to 2.34±0.92 pg·mL^{-1}, ($p<0.001$). All SCB were similarly harmful.

In conclusion, modern farming has not succeeded in making swine confinement buildings inoffensive to exposed subjects.
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Exposure to swine confinement buildings (SCB) constitutes a respiratory health hazard to exposed individuals [1–3]. Swine building workers have a high prevalence of chronic bronchitis and airway hyperreactivity [4–7]. Chronic exposure to this environment causes airway inflammation in most people [8]. Naive healthy subjects exposed for a short period of time develop an acute toxic response manifested by an increase in airway responsiveness and in the number of neutrophils and the levels of pro-inflammatory cytokines in the peripheral blood, the nasal passages, and the lungs [9–13].

The air in swine confinement buildings contains a variety of pollutants that can account for the health effects observed. These include micro-organisms with their endotoxins, gases (NH_3, SO_2), and organic dust (feed, dancers, excrements) [14–17]. It is presently not clear which of these substances or if their combination is responsible for the health effects of SCB. It has recently been shown that nasal filtration did not prevent lung and systemic effects of swine confinement building air, suggesting that small respirable particles are involved [18]. Canola oil spray offers significant protection; the exact effect of spraying oil on the quality of the air in SCB has however not been determined [19].

SCB and swine raising in Quebec (Canada), as for most countries, are not standardized. There is a wide range of building designs and animal raising techniques: different types of feeding, dung collection and elimination, ventilation, heating, cleanliness, etc. Also, the sizes of buildings and the density of pigs raised vary considerably. It is known that the number of microbial contaminants, dust and gas levels vary considerably from one swine building to another [15, 20]. Before generalizing that all SCB constitute a health risk, it is only fair to verify whether different buildings are similarly hazardous and whether modern designs and production techniques have improved or eliminated the risk to exposed humans. Most importantly, if some SCB are safe, or at least safer, while others are more toxic, it will be important to identify the technology that renders them safe or dangerous and isolate what differences in the air composition are associated with different health effects. This information will be useful for understanding what causes the lung response to SCB air and will aid in the design of better swine raising practices.

The purpose of this study was therefore to verify the effects of short term exposure of healthy volunteers to eight different SCB, buildings selected to represent the widest possible range of different buildings and practices in Quebec: dirtiest to cleanest, modern facilities to poorly designed old units, small to large productions. In parallel to the health effects the authors evaluated environmental variables of each building selected.

Materials and methods

Building selection

Eighteen SCB were visited by one of the authors (Y. Cormier) to select the eight buildings included in this study. A preselection, based on farm description and telephone conversations with some producers, having been made from a large number of facilities provided by the Quebec federation of swine producers. Each swine building selected for the study was visited twice between January 13 and March 5, 1997.

Subjects

Eight healthy nonsmoking and nonatopic young males (age 23–28) participated in the study; each subject was exposed once to each of the eight facilities, four volunteers per visit. The exposure consisted of spending 4 h in the SCB, no specific activity was requested; they usually just played cards. All exposures were separated by 1 week. This time sequence was justified by the results of previous studies that showed that the effects of exposure were short lived [9–11]. Volunteers had never been exposed to SCB except for their participation in this study. The protocol was approved by the authors' institution's ethics committee and all participants signed an informed consent form at the beginning of the study.

Physical aspects

Physical aspects were noted for each SCB at the first visit to that building. Parameters noted were: cleanliness (1=clean, 10=dirty), ventilation, air temperature, building size and number of animals. All eight volunteers and the technician who performed the air samplings separately evaluated each building for cleanliness. Air samples were taken to measure total dust and microbiological counts. Bacteria and molds were counted.

Air sampling and analysis

All the samples and measures were taken three times: at the beginning, the middle and the end of the 4-h period, except for dust that was sampled continuously for 4 h. All air samplings were taken on a table, 1 m above the floor. The samplers (AGI filters; Graseby Andersen, Atlanta, GA, USA) were always ~50 cm from the enclosure. No specific study was performed to evaluate whether the site sampled was the most representative, but visually it was the closest to the sources of contamination and as far away as possible from the doors, windows and other ventilation sources.

For dust measurements, preweighed 37 mm polyvinyl chloride (PVC) filters (pore size 0.8 μm) housed in closed-face cassettes were used with SKC 224-44XR personal sample pumps (Dur-Pro; Brossard, Quebec, Canada) calibrated at 1.5 L·min^{-1} (Kurz flow-meter; Instruments Inc., Carmel Valley, CA, USA). The sampling was carried out with the port of entry pointing upwards. Filters were stored in the freezer until the end of the study. Filters were then stored in a drying chamber until constant weight and weighed under controlled atmospheric conditions to avoid rehydration. Control filters were brought to the sampling site, exposed but not subjected to sampling and weighed by the same procedure.

Airborne bacteria were sampled with All-glass impingers-30 (AGI-30; Ace Glass Inc., Vineland, NJ, USA) connected to Gilian Aircon II pumps (Levitt security, Montreal, Canada) at a flow rate of 12.5 L·min^{-1} for 16 min, three times during the 4-h period. Pump flow rate was set with a Kurz flow-meter (Instruments Inc.). Sterile AGIs contained 20 mL of sterile saline water (0.8% NaCl) were kept on ice after the sampling. Back at the laboratory (maximum 1 h after the sampling procedure), the sample volumes were measured (to evaluate evaporation) and completed at 30 mL with sterile saline water containing 0.15% Tween 80 (final concentration of ~0.05%) and diluted to 10^{-2}. Nondiluted and diluted samples were plated in triplicate on Trypticase soy agar (TSA) (Difco, Detroit, IL, USA) containing cycloheximide (500 mg·L^{-1}) to avoid mold growth and incubated at 30°C for 60 h. Total bacteria were counted at the dilution where the plates showed between 30–300 colonies. As a standard procedure the authors had planned to do control outside samplings, about 1 km upwind from the SCB, when the outside temperature was above -4°C and compare the levels to the indoor values. Any outside colonies obviously similar to inside ones would be subtracted from the inside values. Since this study was performed during the winter, outside sampling was only carried out once (temperature=-2°C) and no growth was seen.

AGI-30 samples were used to determine airborne endotoxin concentrations. Samples were kept frozen (-20°C) in plastic tubes before measurement. Endotoxin was measured with Limulus Amoebocyte Assay (LAL) endpoint chromogenic test (Associates of Cape Cod, Woods Hole, MA, USA). Controls were obtained with sterile saline water containing 0.05% Tween 80 with which sterile AGI-30 samplers were washed for a few minutes. This procedure allowed the measurement of the initial contamination of the samples and material by endotoxins. The control values were subtracted from the sample values. Inhibition and enhancement tests were also performed on the samples.

Airborne molds were sampled with 6-stage Andersen impactors (Grasby Andersen) connected to Gilian Aircon II pumps (Levitt security) at a flow rate of 28.3 L·min^{-1} for 2 min, three times during the 4-h period. Pump flow rate was set with a Kurz flow-meter (Instruments Inc.). Andersen samplers were loaded with Rose-bengal agar (Difco) containing chloramphenicoi (50 mg·L^{-1}) to avoid bacterial growth. Dishes were incubated at 30°C for 5 days. Molds were identified with microscopic and macroscopic observations. Control sampling was performed as for the bacteria. The control samples were used in comparison with the indoor samples. If similar mold colonies were found outside and inside, the outside level was subtracted. If the population was different, the controls were not used.

The 4 h ammonia samplings were performed in triplicate. Sulfuric acid pretreated silica gel columns (Dur-Pro) were used with a low flow rate pump (0.15 L·min^{-1}) calibrated with a SKC UltraFlo Electronic Calibrator (Dur-Pro). Ammonia was analysed by chromatography in a reference laboratory. Control columns were brought to the

sampling site, exposed to the ambient environment but without pump sampling and were analysed by the same procedure.

Subjects' evaluations

The volunteers were evaluated at baseline and before and after each exposure. Baseline evaluation included: nasal lavage, venous blood samplings, and methacholine bronchoprovocations. Nasal lavage and blood sampling were carried out at 15:00 h on the first baseline day, the methacholine challenge performed the following morning at 08:00 h. For each subject and each exposure, forced expiratory flows were obtained at 09:00 h and 15:00 h (before and after exposure), nasal lavage and blood sampling were obtained at 15:00 h and methacholine challenges in the morning (08:00 h) the day following the exposure. Subjects whose provocative concentration causing a 20% fall in forced expiratory volume in one second (FEV1; PC20) dropped after exposure were retested the morning before being sent to the next SCB. Also, the pre-exposure forced expiratory flows were obtained to ensure that all subjects were free of airflow obstruction before going into the SCB.

Lung functions

Forced expiratory flows and volumes were obtained using a Vitalograph spirometer (Roxon, Buckingham, Buckinghamshire, UK). Complete flow curves, from which the forced vital capacity (FVC), FEV1 and the ratio FEV1/FVC were derived. The tests were performed according to the American Thoracic Society standard procedure [20]. For the methacholine challenges only FEV1 measurements were obtained.

Standard methacholine challenge procedure as described by JUNIPER *et al.* [19] was used in this study. Briefly, doubling doses of methacholine were delivered *via* a calibrated Wright nebulizer (Roxon Meditech, Montréal, Quebec, Canada) 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. PC20 was obtained by intrapolation on a semi-logarithmic scale.

Nasal lavage

For this sampling, after blowing and wiping their nose, the subject was instructed to occlude their posteriopharynx by positioning the tongue against the soft palate. They then tilted their head backward and 5 mL of 0.9% warm saline was instilled into each nostril. The saline wash was kept in the nasal cavities for ~20 s while the subject held their breath and maintained a closed posterio-pharynx and a backward tilted head position. The subject then tilted their head forward and blew the nasal wash into a clean dry flask [22].

The nasal fluid recovered was measured and centrifuged at 500 × g for 10 min at 4°C. The supernatant was aliquoted and frozen at -70°C for subsequent interleukin(IL)-8 analysis. The cell pellet was resuspended in 100 μL to 1,000 μL of Hanks' balanced saline solution (HBSS) for total cell count. Differential counts were performed on Diff-Quik stained glass cover slip slides [23]. Cell viability was verified by trypan blue exclusion.

Blood samples

Ten millilitres of heparinized venous blood was withdrawn. Total and differential counts were obtained electronically with a cell counter STKS (Courter Electronics Ltd, Hialeah, FL, USA). Another 10 mL of nonheparinized blood was withdrawn, allowed to clot and the serum separated by centrifugation. Serum was kept frozen at -70 until processed for the measurement of IL-6 levels.

Cytokine (interleukin-8 and interleukin-6) measurements

Levels of cytokines in nasal lavage fluids and sera were assayed by high sensitivity immunoassay kits from PerSeptive Diagnostics (Cambridge, MA, USA) for IL-8 and from R & D Systems Inc. (Minneapolis, MN, USA) for IL-6.

Statistical analysis

The statistical method used to perform the comparisons between SCB was a one-way ANOVA with a factor representing the group effect (the comparison between the eight buildings). FEV1 and FVC were analysed for any changes between morning and afternoon. Normality and variance assumptions were tested. *Posteriori* comparisons were performed with Duncan's method. Significant differences between values are expressed with letters, SCB with a same letter were declared not significantly different. Relationships between parameters are expressed with the Pearson's correlation coefficients as the linearity between these parameters were observed. The results were considered significant if p-values were ≤0.05. The data were analysed using the statistical package program SAS (SAS Institute Inc., Cary, NC, USA).

Results

Building variables

As per the experimental design there was a wide range of cleanliness scores between the eight SCB visits (table 1). Table 1 also shows that there were large differences between buildings sizes and number of pigs per operation (108–834), amounts of airborne dust (2.20–5.62 mg·m^{-3}), number of bacteria (167 × 103–929 × 10^3 CFU·m^{-3}), endotoxin levels (215–596 Eu·m^{-3}), molds (138–1805 CFU·m^{-3}) and ammonia (2.8–38.6 parts per million (ppm).

Lung functions

Individual values for FEV1 and FVC before and after each exposure are given in figure 1. There was a significant fall in both FEV1 and FVC after the exposures to each SCB (all p≤0.0002). There were no differences between the SCB. PC20 fell by two or more concentrations after exposure in only 3/64 instances. The decreases reached statistical differences only in SCB No. 2 (p= 0.04).

Table 1. – Environmental data for each of the eight swine confinement buildings visited

Building No.	Visual score	Building size m^3	No. of pigs	Dust $mg \cdot M^3$	Bacteria $CFU \cdot m^3$	Endotoxins $EU \cdot m^3$	Molds $CFU \cdot m^3$	NH_3 ppm
1	1.38	2100	834	2.53	929000	596	532	21.83
2	6.75	816	300	2.85	523000	296	1802	2.80
3	8.25	523	350	4.31	167000	281	1805	8.10
4	4.50	1920	800	5.62	481000	536	954	37.00
5	1.50	1226	363	2.20	239000	215	138	27.00
6	5.88	1223	108	3.32	218000	448	595	38.55
7	5.38	799	364	3.33	298000	338	954	19.62
8	2.37	1579	665	3.35	692000	523	282	10.80

The values for dust, bacteria, endotoxins, and molds are the means for two visits; sample performed in triplicate at each visit. CFU: colony forming units; EU: endotoxin units; ppm: parts per million.

Nasal lavage and blood analyses

Neutrophils were significantly increased in the nasal lavages after exposure to each SCB except building No. 6 where the p-value reached 0.06 (fig. 2a). White blood cells increased after each exposure (p-values 0.02–0.001) (fig. 2b). Nasal levels of IL-8 were highly increased after all exposures with p-values ranging 0.003–0.0001 (fig. 3a). Blood levels of IL-6 were also always highly increased after exposure with p-values 0.01–0.0001 (fig. 3b). There were differences between different SCB for all of these parameters (see figs. 2 and 3). The differences were not consistent but were spread out among the different SCB.
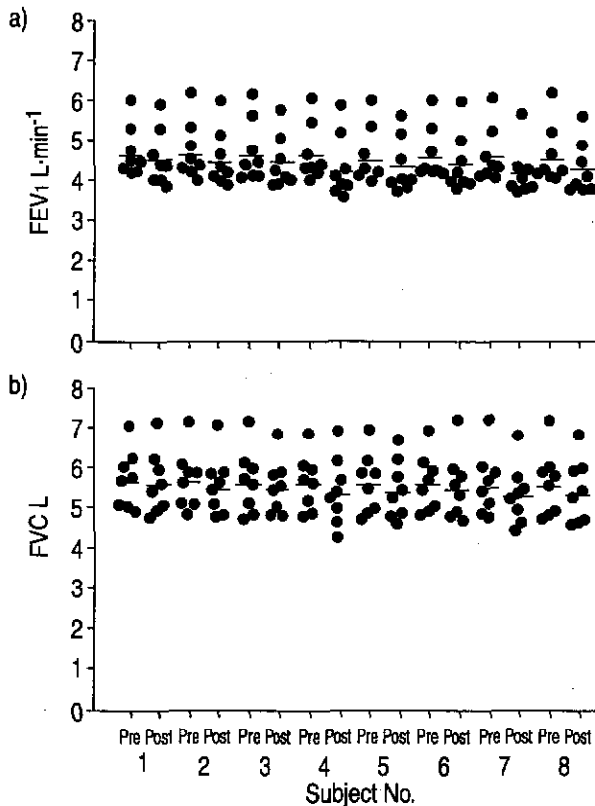

Fig. 1. – Individual values and means of a) forced expiratory volume in one second (FEV1) and b) forced expiratory vital capacity (FVC), for each of the eight subjects before and after exposure to each of the eight swine confinement buildings visited. Both parameters were similarly and significantly decreased after exposure for each of the eight buildings.

Correlations between variables

The contingency table 2 summarizes the relations between human and environmental variables. The only significant correlation between building cleanliness and environmental parameters was between NH_3 and IL-6 in the serum. Since all possible pairs of variables were tested, the significance of a 0.04 p-value remains doubtful.
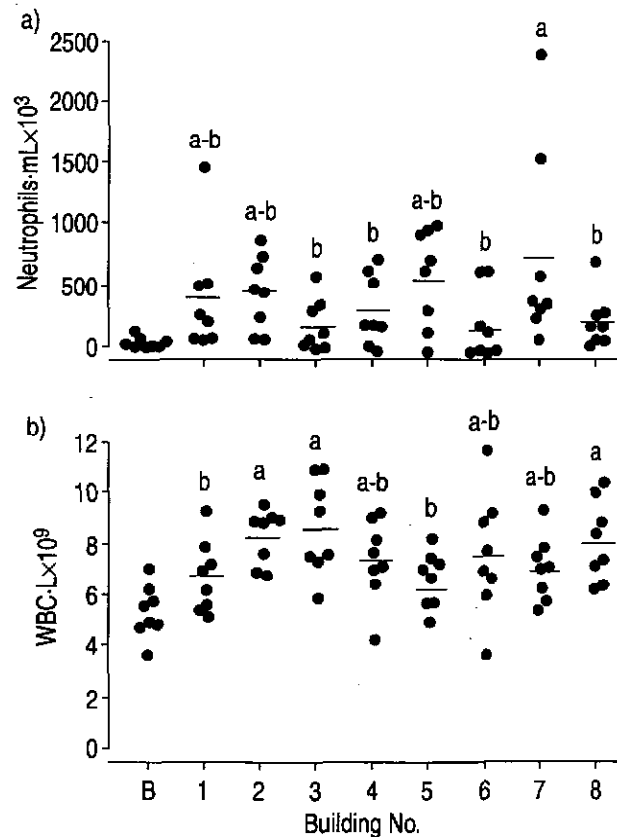

Fig. 2. – Individual values and means a) for the number of neutrophils recovered by nasal lavage and b) white blood cells (WBC). These cells increased from baseline for all but one building (No. 6) for nasal neutrophils where the p-value reached 0.06. For clarity the significance between baseline values and exposure days are not shown on the graph. Differences between different buildings are represented by different letters above the data. In other words, all buildings with an "a" above the data are similar to each other and different from those with a "b" primes. For example, results of neutrophils for building 7 are different from those of buildings 3, 4, 6, and 8. B: baseline.
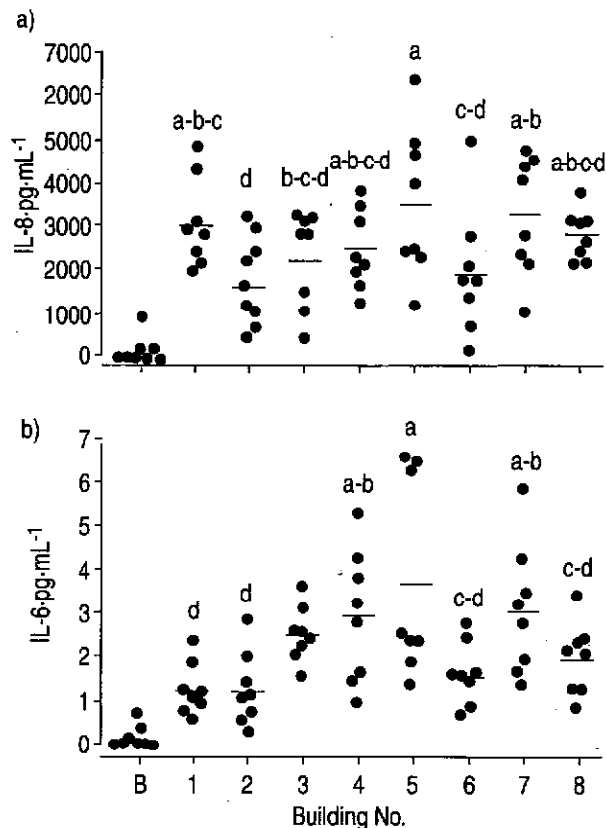
Fig. 3. – Individual values and means a) nasal levels of interleukin(IL)-8 in picograms per millilitre and b) blood levels of IL-6. Highly significant and similar increases were seen after each swine building exposure. Again, as identified by different letters, some buildings had a greater effect than others. B: baseline. The differences between the buildings are indicated by the letters "a, b, c and d". Buildings marked "a" are similar to other buildings marked "a" but are different from buildings marked with any other letter. Buildings marked "a-b", for example, are similar to all buildings marked "a" and to all buildings marked "b".

Discussion

Health effects of a short term exposure of naive subjects to an SCB in this study are less striking than what has previously been described [9–12]. For example, in the current study very little and mostly nonsignificant changes in airway responsiveness were seen. These differences can be explained by the designs of the different studies. In previous reports volunteers were exposed for longer periods and performed physical activities while inside the buildings or were actively working weighing pigs [10, 12, 13]. In the current study, subjects only spent 4 h inside the SCB and were not asked to do any specific activity. The exposure in this study was, however, sufficient to elicit significant effects on lung functions and induce inflammatory responses as confirmed by the increases in neutrophils and in pro-inflammatory cytokines in both the nasal wash and the peripheral blood. The light exposure in this study was purposely used in order to minimize the risk of having cumulative effects of the SCB exposures on airway functions as was previously described [10, 12]. To further assure that there was no carry over from building to building, all subjects who had a fall in PC_{20} methacholine of one concentration or more were rechallenged before being exposed to the next SCB and a measure of forced expiratory flows was obtained before each exposure. In all subjects in whom control bronchoprovocation was required the PC_{20} had returned to baseline values before the re-exposure (data not shown). All FEV_1 and FVC values had also returned to baseline values before each re-exposure. Previous studies have documented that the inflammatory responses to SCB exposure were of short duration [9, 11]; these parameters were therefore not remeasured before each visit in the current study. The authors therefore show that low level exposure can be safely repeated at weekly intervals without causing alterations in lung functions and airways responsiveness.

Since the SCB were visited in random order in terms of their cleanliness and other environmental parameters the authors do not believe that tachyphylaxis to repeated exposures could explain the findings.

The changes in FEV_1 and FVC, showing a decrease in the afternoon values compared to those in the morning, are probably underestimated since the normal circardian rhythms would have favoured the opposite [24]. These changes in lung function did not translate into significant differences in airway responsiveness as measured the day

Table 2. – Correlation between environmental parameters and human data

		ΔFEV_1	ΔFVC	Neutro	WBC	IL-8	IL-6
Visual score	R	0.23	0.12	-0.18	-0.00	-0.46	-0.26
	p	0.59	0.77	0.67	0.99	0.26	0.55
No. of pigs	R	0.08	0.08	0.09	-0.61	0.62	0.21
	p	0.85	0.85	0.84	0.11	0.10	0.62
Dust	R	0.07	-0.24	0.47	-0.18	0.37	0.36
	p	0.87	0.57	0.24	0.68	0.36	0.38
Bacteria	R	0.36	0.40	-0.13	-0.43	0.42	-0.35
	p	0.38	0.32	0.76	0.28	0.30	0.40
Endotoxins	R	-0.01	-0.07	0.25	-0.61	0.72	0.15
	p	0.99	0.87	0.55	0.11	0.05	0.71
Molds	R	0.30	0.19	0.19	-0.05	-0.44	-0.30
	p	0.47	0.66	0.65	0.90	0.27	0.47
NH_3	R	-0.29	-0.22	-0.21	0.43	0.51	0.74
	p	0.49	0.60	0.62	0.28	0.20	0.04

The only significant correlation found was between the serum levels of interleukin (IL)-6 and ammonia. ΔFEV_1: change in forced expiratory volume in one second; ΔFVC: forced vital capacity; Neutro: number of neutrophils in nasal lavage; WBC: white blood cells; IL-8: interleukin-8 in nasal lavage; IL-6: serum levels of IL-6; p: probability; r: relationship. Pearson's correlation coefficients were used to assess the relationships between the parameters.

following each exposure suggesting that any inflammation or oedema were of short duration.

Of particular interest, no SCB was totally devoid of inflammatory effect. Modern technology in swine raising and cleanliness inside the buildings therefore still fails to render the air in this environment harmless to human beings. The environmental samplings and the parameters that were measured in this study were not sensitive enough to explain the differences in the effect of each building on different human variables.

In conclusion therefore: 1) repeated short term exposure to swine confinement buildings induces a reproducible, significant, and transient lung and systemic inflammatory response; 2) with participants at rest during exposure, the effects of swine building environment are transient and not cumulative and do not induce significant bronchial hyperreactivity; 3) visibly cleaner modern facilities are not less harmful than older ones; and 4) visual aspects, number of pigs raised and routine spot check air analysis are not useful in evaluating the potential toxic effect of inhaling swine building air.

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