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SEASONAL VARIATIONS IN WORK-RELATED HEALTH EFFECTS IN SWINE FARM WORKERS

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Abstract: The aim of the project was to investigate whether there were diminished health effects in swine farm workers during summer compared with winter, as seasonal differences in concentrations of bioaerosols have been reported. Twenty-four workers were visited once during each season. Before and after a work shift, they underwent lung function testing and blood sampling. During work, they wore personal air sampling equipment. The mean endotoxin exposure of the workers was highest during winter (25,690 *vs.* 6,553 EU/m^3; p = 0.004). Although exposures to endotoxin and CO_2 varied between the seasons, no differences in lung function were found between them. White blood cell concentration increased over the work shift from 5.74–6.82 in winter (p < 0.0001) and from 5.80–6.38 in summer (p = 0.014). These increases differed between the two seasons (p = 0.032). Plasma tumour necrosis factor concentrations fell over the work shift only during winter (1.34–1.24 pg/ml (p = 0.03) (p = 0.014 for the difference between seasons). Plasma interleukin-6 increased over the work shift independently of season (p = 0.0006). The study supported our hypothesis of adverse effects on lung function and immune system, but less so during summer than during winter among Québec swine farm workers.

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INTRODUCTION

In modern swine production with animals concentrated in large operations, workers are usually exposed for several hours every day to an indoor work environment with high concentrations of potentially harmful airborne agents. Dust generated by the animals contains particles of stools [22] and feed [5]. Gases are emitted from swine manure pits or from underneath slatted floors [9]. Adverse effects on lung function from such exposures are well described in several studies. Swine confinement buildings are ventilated to minimize the concentrations of gases and airborne agents. However, ventilation rates are not high enough to completely clean the air [1, 21]. In cold weather, ventilation rates are set to control air moisture level and to save heat. In warm weather, ventilation rates are maximal in order to keep the temperature down. Logically, in climates with cold winters and hot summers it is commonly assumed that indoor air in swine farms is much cleaner during summer than during winter. This has been confirmed in studies in Québec, in particular with regard to gases and dust [4, 11, 15]. Consequently, the exposure of workers to airborne pollutants in the swine buildings should also differ between seasons. Most earlier studies on health effects of swine farm exposure do not include exposure measurements; the studies that do, have been performed only during winter or

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during activities generating high levels of exposure (e.g. personal dust exposures around 7 mg/m^3 in [6, 17]). Studies on health effects of lower levels of dust exposures in swine farming are scarce. Effects on serum cytokines were reported in a study with mean inhalable dust exposures of 1.23 mg/m^3 [14], but no lung function decreases were observed in another study with personal exposures of 1.57 mg/m^3 [13]. In the latter paper, which is the only paper on cross shift lung function changes among swine farm workers on different days of the week that we have found, no differences were observed between weekdays [13]. To our knowledge, no studies have investigated possible exposure related differences in cross shift health effects between summer and winter in the same swine farm workers.

The purpose of the study was to compare the responses of the lungs and of the immune system to the work environment of swine farm workers across a workshift during winter with the responses during summer. We hypothesized that due to differences in exposure: 1) the workers would be adapted to their work environment and experience limited health effects, and 2) the adverse health effects caused by a day's work would be reduced during summer compared with winter.

MATERIALS AND METHODS

Selection of workers. Swine farm workers were identified preferably within one hour of driving distance from Quebec City with the help of personal contacts from previous studies, visits in the relevant areas, local telephone registers, and registers of pork producers. Swine farms were of varying size and age. They all included at least one mechanically ventilated finishing building. We visited only finishing operations since previous studies in Québec have shown that these have higher levels of organic dust than farrowing buildings [4]. All studied subjects are referred to as workers, although some were themselves the owners of the swine farm operations. Males and females with at least 6 months experience in swine farm work were eligible for inclusion. Smokers and workers suspected of asthma or other lung diseases were excluded from the study. The workers were contacted by telephone or visited, and if they showed interest in the project, a longer visit was scheduled approximately 1–2 weeks before the first evaluation. During these visits, two of the investigators presented and explained the project in more detail to the pork producer and interested workers and answered questions. Complete spoken and written information on the project was given immediately prior to the first evaluation, and the worker signed an informed consent to participate. Ten healthy hospital staff agreed to participate as controls. After explanation of the project and signing of informed consent, they underwent spirometry and blood sampling early and late during one normal workday at their workplace. The project was approved by the institutional Ethics Committee at the Laval Hospital.

Visits. Two visits were planned for each worker: The winter visits took place between 18th October and 26th April, the summer visits between 30th May–14th September. The project was conducted in 2005 and 2006. The gap between visits varied from 2.5 to 9.5 months. Both evaluations were planned to take place after a period of continuous work in the farm of at least 4 days (in most cases, Tuesdays after a week and a week-end at work). The first evaluation was performed in the morning immediately prior to the first entry of the worker into the animal house. In a few cases where the worker had to enter the building before the morning evaluation, he/she was asked to wear N95 respirators (3M, St Paul, MN, USA) for protection of the airways. The 2nd evaluation was performed in the afternoon at the end of the work shift, and preferably at least 6 hours after the beginning of the work in case of short work shifts. During 15 of the 41 evaluations the worker did not spend 6 hours in the building, and the 2nd evaluation had to be performed between 248 min and 359 min after the start of the work. The evaluations were performed at the work place, either in the home of the worker or pork producer or in an office adjacent to the swine buildings when such an office was available.

Exposure assessments. On visit days the workers were equipped with personal samplers and instructed to carry them from entry into the swine houses until end of work within these houses. Glass fibre filters were used for sampling of endotoxin and preweighed PVC filters (0.8 mm) for total dust sampling. Filters were housed in individual closed-face 37-mm cassettes (SKC Inc., Eighty Four, PA, USA). The cassettes were attached to GilAir-5 sampling pumps (Sensidyne Inc., Clearwater, FL, USA) calibrated in the morning and set at a flow of 2 L/min. The worker carried four such pumps as sampling was run in duplicate for both endotoxin and dust. Control filters were brought to the sampling site and exposed, but not subjected to sampling, and assessed by the same procedures as filters subjected to sampling. All glass filters were stored at -20°C until the end of the study. These filters were extracted in sterile PP tubes (Sarstedt Inc., Newton, NC, USA) in 20 ml pyrogen-free saline containing 0.02% Tween 20 and extraction solutions vortexed for 1 hour. The solution was then spinned for 5 minutes at 1,500 RPM and the supernatant collected, aliquoted, and stored at -20°C until analysis. Endotoxin measurements were performed in duplicate for each filter using the endpoint chromogenic LAL assay (Associates of Cape Cod, Woods Hole, Mass., USA), as previously described [11]. Endotoxin concentrations were calculated in EU/ml and corrected for concentrations on the unexposed control filters by subtraction of these. PVC filters were first placed in a drying chamber for 24 h, then in a controlled atmosphere (25°C and 45% RH) for 24 h and weighed in this atmosphere. The gas concentrations were measured inside a rearing facility and averaged over 1 h in the mornings on each visit: NH_3 with a Toxi Ultra sensor (Biosystems

Middletown, CT, USA) and CO_2 with a Q-Track plus model 8552 (TSI, St-Paul, MN).

Workers' evaluation. During the first visit, a history based on a questionnaire derived from the standard American Thoracic Society (ATS) [12] questionnaire for respiratory diseases with additional questions on the current job, use of respiratory protection, job history, dust and gas exposures, was taken for each subject. A Tanita TBF-215 Body Composition Analyzer (Tanita Corp., Tokyo, Japan) was operated according to the manufacturer's instructions to obtain height, weight, and body mass index (BMI). At the 2nd visit, only questions on respiratory protection, medication, and work in the previous seven days were repeated.

Lung function measurements. All workers performed spirometry before and after work, following ATS guidelines [23] with a MIR Spirobank G and a computer equipped with WinspiroPRO 1.1.6 software (Medical International Research, Rome, Italy) with the help of a trained nurse or medical doctor. It was always performed standing and without a nose clip. Some of the younger workers were unable to exhale for at least 6 sec; their results were included despite this. The best of at least 3 acceptable measurements were used at each examination. Antibacterial/viral filters (DCII filter, Ferraris Respiratory, Louisville, CO) were used. The spirometer was calibrated before and after the study with a 3.0 L syringe at different flows, and no change in accuracy was observed.

Blood samples. Venous blood samples were taken before and after work at each visit in K3 EDTA-coated tubes (either from BD Vacutainer, Franklin Lakes, NJ, USA or Greiner bio-one, Monroe, NC, USA). One tube was kept at room temperature for white blood cell count. At the end of each clinical evaluation, the remaining tubes were centrifuged for 10 min at 1,200 g in a Medilite 6 (Thermo Corporation, Milford, MA, USA). The plasma was transferred to a Sarstedt screw cap tube conical 15 mL (Sarstedt Inc., Newton, NC, USA), mixed gently, pipetted in aliquots of 600 µL, and finally frozen on dry ice. Upon return to the laboratory after the final evaluation of the day, the samples were stored at -80°C. One tube was used for white blood cell counts were determined using a Coulter counter at the Laval Hospital laboratory (Coulter Electronics of Canada, Burlington, Canada). C-reactive protein (CRP) was measured by immunonephelometry with a High Sensitivity CRP reagent on a BNProSpec (Dade Behring, Marburg, Germany) by the Biochemical Service at the Laval Hospital. At the end of the study, the concentrations of Tumour Necrosis Factor (TNF), TNF-receptor Type A (TNF RII), interleukin-6 (IL-6), and soluble L-selectin (CD62L) were measured with commercially available chemiluminescence enzyme-linked immunosorbent assay kits from R&D (R&D Systems, Minneapolis, MN, USA). The mean limits of detection were 0.12 pg/ml for TNF, 0.6 pg/ml for TNF RII, 0.039 pg/ml for IL-6, and less than 0.3 ng/ml for CD62L.

Statistical methods. Means or medians are reported as appropriate depending on the distribution of the data. Accordingly, either Wilcoxon signed rank or Students T-test were used for group comparisons. Pearson Chi^2 test was used for comparing gender distribution between groups. Repeated measures were analyzed using mixed models. Subjects were treated as random block effects. The statistical approach used was to perform a multivariate repeated measures design (doubly multivariate data) with a fixed factor linked to the seasons (summer versus winter) and the other fixed factor to the visits (morning versus afternoon). The unstructured@ar(1) covariance structure was used for the analyses with a general Kenward-Roger approximation for the denominator degrees of freedom. The variance assumptions were verified using the Brown and Forsythe's variation of Levene's test statistic. The univariate normality assumptions were verified with the Shapiro-Wilk tests. The multivariate normality was verified using the Mardia's test. Logarithmic transformation as well as the arcsinus of the square root transformation was used to achieve these assumptions. The results were considered significant with p-values ≤ 0.05. The repeated measures analyses were conducted using the statistical package SAS v. 9.1.3 (SAS Institute Inc, Cary, NC, USA). SPSS v. 13.0 was used for the remaining analyses. The environmental exposure variables were included as covariates in the repeated measures analyses. A sensitivity analysis was performed by running the repeated measures analyses after exclusion of data from days with less than 3 h of swine exposure – corresponding to the lower quartile of swine exposures for the workers.

RESULTS

Of the twenty-four swine farm workers included, seven were not visited during summer: two because of job change; four declined continued participation; and one was included in the winter study after the summer visits had been performed. Ten non-exposed controls were included.

The demographic data on the swine farm and controls are listed in Table 1. Subjects were mostly males with a mean age of 39–41 years and mean BMI of 25–27. The two groups only differed in height, the controls being taller than the swine farm workers ($p = 0.046$). Within the swine farm workers, the length of employment varied considerably, whereas all reported a fairly high number of hours spent with swine per week in their current job. Of the swine farm workers, 38% reported some sort of respiratory symptoms that improved after time off work, and 25% of them reported that they sometimes used respiratory protection.

As shown in Table 2, the time from the beginning of the work shift to the 2nd clinical evaluation and duration of work did not differ between the two visits. Only temperature, airborne endotoxin, and CO_2 differed significantly between

Table 1. Demographic and work characteristics of the study subjects.

	Swine farm workers	Control workers
Number (males : females)	24 (22 : 2)	10 (8 : 2)
Age (years)	40.8 (20; 69)	39.0 (21; 56)
Height (cm)	171.5 (157; 186)	176.7 (160; 184)
Weight (kg)	78.5 (54; 123)	76.9 (60; 96)
Body mass index (BMI)	26.5 (20.9; 38.8)	24.8 (17.7; 33.2)
Years working with swine	16.0 (0.7; 50)	0
Work hours on swine farm/week	41.2 (28; 60)	N/A

Age, height, weight, BMI, time working with swine and work hours are all means. N/A – not applicable.

summer and winter (p-values: 0.012, 0.004, and 0.012 respectively).

The lung function indices: one-second forced expiratory volume (FEV_1), forced vital capacity (FVC), the ratio between the two, peak expiratory flow (PEF), forced expiratory flow (FEF) at 25%, 50%, 75% of expired volume, and maximal mid-expiratory flow are given in Table 3. No differences between winter and summer were found in any of these lung function indices, neither in the mornings nor after the work. Nor were there any cross shift changes in any of the lung functions indices.

Blood leukocyte concentration and some of their subpopulations showed cross shift changes during both the winter and the summer visits, as shown in Table 4 and Figure 1. Leukocyte concentration increased over the work shift from 5.74–6.82 during winter ($p < 0.0001$) and from 5.80–6.38 during summer ($p = 0.014$) and these increases differed between the two seasons ($p = 0.032$ for an interaction between time and visit). At both visits, the blood neutrophils increased with time, both in terms of concentration ($p < 0.0001$) and in percent of total leukocytes ($p = 0.0014$) (Fig. 2). Basophils increased in concentration over the work shift only in winter (from 0.38–0.42%; $p = 0.007$) and this interaction between visit and time was statistically significant ($p = 0.025$). Finally, monocyte concentration did not differ between visits, but showed a cross shift decrease in percent of total leukocytes ($p = 0.05$).

Plasma TNF concentrations decreased over the work shift during winter from 1.34–1.24 pg/ml ($p = 0.03$), but not during summer when they remained at approximately 1.35 pg/ml ($p = 0.014$ for an interaction between visit and time) (Fig. 3).

Plasma TNF-RII concentrations also decreased over the work shift. As for TNF, this decrease was only observed during winter (with $p = 0.005$). A p-value of 0.054 for an interaction between visit and time indicates that it is likely that changes in TNF-RII differed between winter and summer.

Plasma IL-6 increased over the work shift ($p < 0.0001$) (Fig. 4). During winter, the increase was from 0.9–1.24 pg/mL ($p = 0.0006$) and during summer from 1.03–1.73 pg/mL ($p = 0.0006$), with no statistically significant difference between the two visits.

In the mornings, prior to any work exposure, none of the inflammatory indices differed between the winter and the summer visits (data not shown).

Of the environmental exposure variables, only indoor temperature and NH_3 had significant effects in the mixed model, and this effect was limited to increased FEF_{50} and MMEF with lower temperature or higher NH_3 (p values between 0.036–0.049). None of the remaining environmental exposure variables (RH, CO_2, endotoxin, and dust) had any effects on health outcomes in the repeated measures analyses (Tab. 5). All environmental exposure variables did, however, affect the models and change some of the relations observed without them. This was most pronounced for temperature, the inclusion of which made changes in FEV_1 and MMEF with visit appear as well as interactions between visit and time for FEF_{50}, MMEF, and PEF. However, these effects appeared to be entirely caused by the more restricted selection of health data induced by inclusion of temperature in the model: When performing the statistical analyses of data only from days with valid temperature information, but without temperature in the model, the same changes in lung function indices appeared.

Some changes in the main outcomes appeared when testing the quality-restricted subsets of data in the sensitivity

Table 2. Work conditions during the two study visits.

	Summer visit	Winter visit	P-value
Duration from workstart to 2nd clinical evaluation (min)	373 (249; 482)	371 (269; 553)	0.55
Length of workday (min)	238 (41; 389)	235 (60; 436)	0.55
Personal dust exposure (mg/m^3)	2.39 (0.61; 10.24)	3.80 (1.3; 7.8)	0.39
Personal endotoxin exposure (EU/m^3)	6553 (2218; 25861)	25690 (1800; 69096)	0.004
Area per animal (m^2/swine)	0.91	1.08	0.59
Indoor temperature (°C)	25.0 (19.3; 29.3)	20.3 (15.2; 20.7)	0.012
Relative humidity (%)	73.3 (58.9; 88.6)	61.5 (51.7; 92.1)	0.16
CO_2 (ppm)	787 (478; 1348)	2276 (1528; 4158)	0.012
NH_3 (ppm)	9.7 (5.3; 41.2)	12.0 (3.3; 45.2)	0.37

Durations are means; exposures and animal densities are medians. Ranges are given in parentheses.

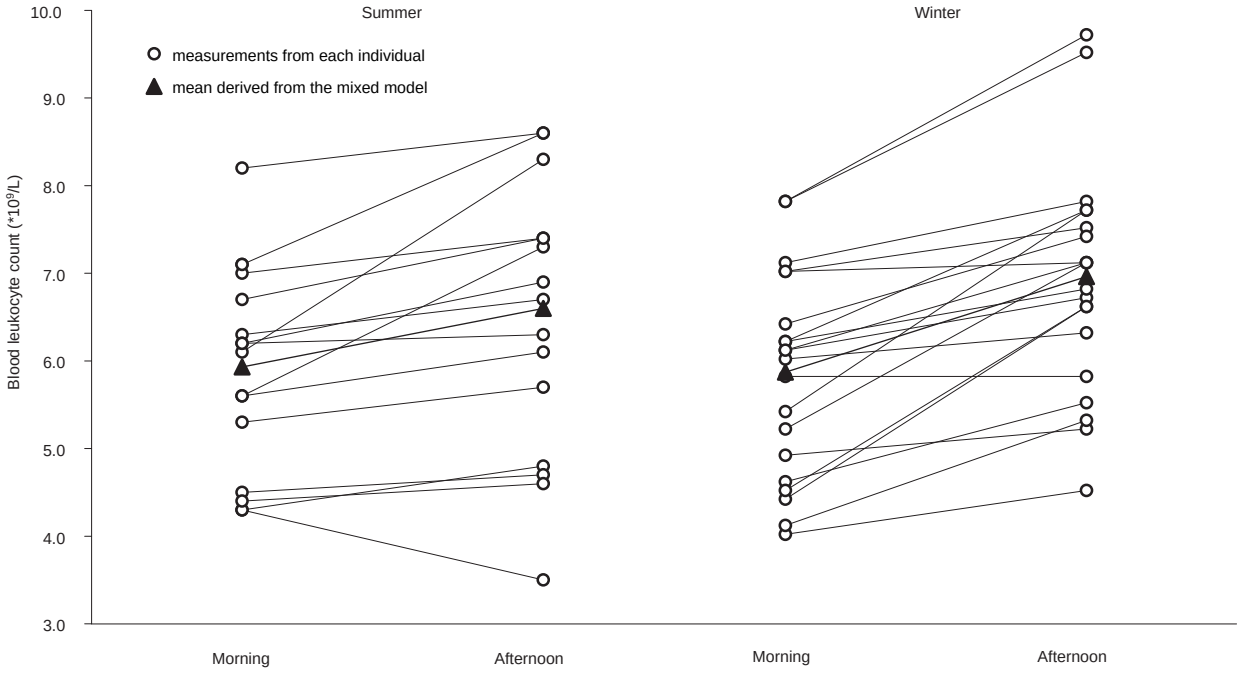

Figure 1. Cross shift changes in blood leukocyte counts during summer and winter in the 24 visited workers.

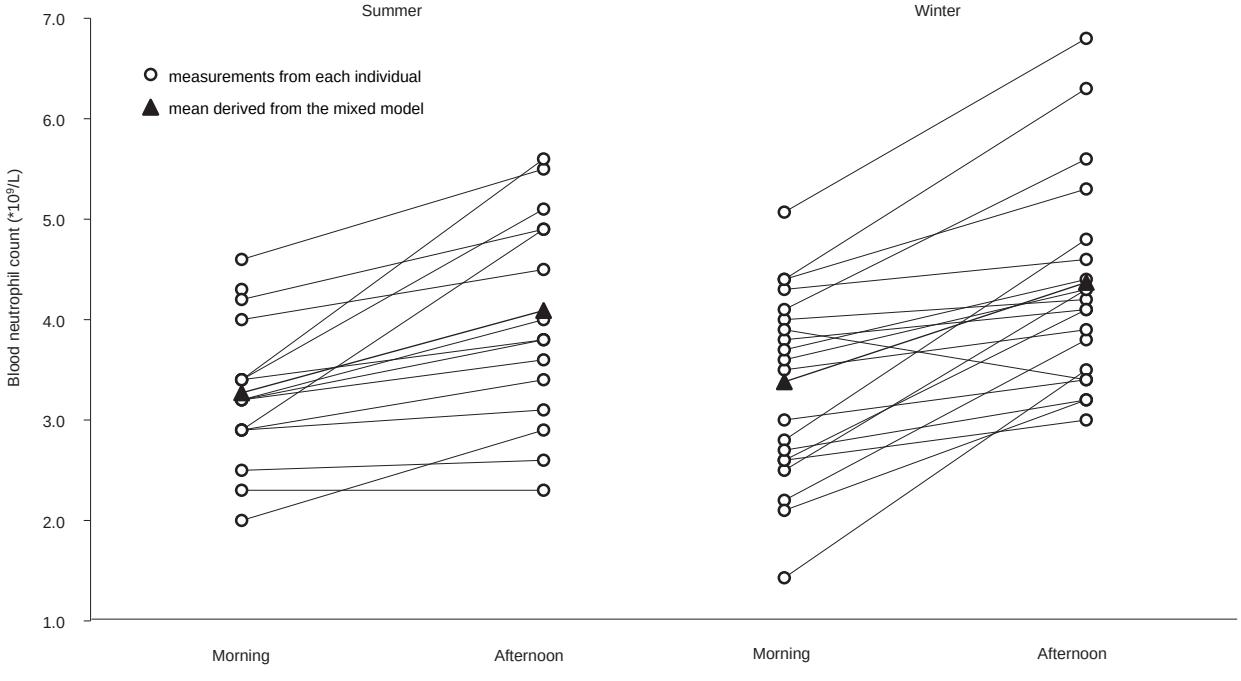

Figure 2. Cross shift changes in blood neutrophil counts during summer and winter in the 24 visited workers.

analysis. Two visit * time interactions revealing cross shift declines in FEV_1 ($p = 0.007$) and increases in PEF ($p = 0.003$) during winter, but not during summer, appeared when excluding days with less than 3 h of swine exposure. The latter also appeared when excluding visits where the worker had entered animal buildings before the 1st evaluation of the day ($p = 0.013$). When excluding days with less than 3 h of work, or with only work in farrowing buildings FEV_1, FEV_1/FEC and MMEF all showed cross shift decreases during winter in contrast to increases during summer (p-values between 0.008–0.04 for this interaction). The visit * time interaction of the blood leukocyte concentration disappeared when restricting to days with at least 3 hours of work or not spent in farrowing units, but was robust to the other restrictions applied. No matter what the selection, the interaction between time and visit remained close to statistically significant for TNF (although with $p = 0.07$ for days with at least 3 h of work), but disappeared for

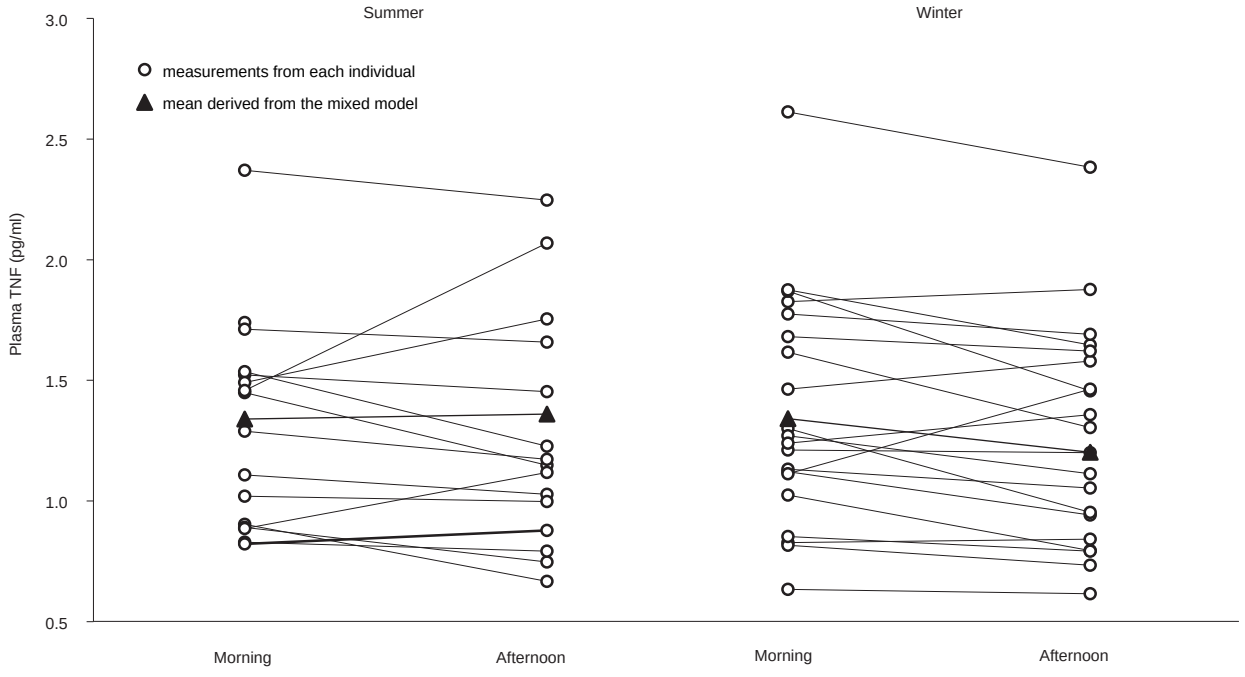

Figure 3. Cross shift changes in plasma TNF concentration during summer and winter in the 24 visited workers.

TNFRII. The cross shift changes in neutrophils and in IL-6 remained no matter what the selection.

As shown in Table 3, the PEF increased among controls as compared to a decrease among the workers ($p = 0.005$).

DISCUSSION

The most striking finding of this study was that cross-shift health effects were very mild in this group of regularly exposed swine farm workers, even during the supposedly high exposure winter visits. In view of the many studies reporting cross shift declines in lung function (e.g. [10, 16, 17, 26]), it is remarkable that no such declines were observed in this study. However, the normal circadian increase in lung function from morning to evening was absent; indicating possible adverse effects of the swine farm work exposure, despite the lack of lung function decrements. When excluding the less exposed workers in the sensitivity analysis, such cross shift declines appeared; indicating an obstructive decrease in lung function during winter. Taken together, these observations support the hypotheses that daily exposure to swine causes adaptation reducing but not omiting effects during the heavy exposure season.

The observation of minimal effects on the white blood cells over the workday contrasts with the results of a number of studies on previously unexposed (naïve) volunteers in whom large changes in white blood cell counts as well as in lung function occurred [2, 3, 24, 25]. Compared with these studies, we found limited cross shift increases

Table 3. Lung function measurements before and after work shift during the two seasons in swine farmers.

	Summer visit		P-value	Winter visit		P-value	P (interaction)[a]
	Morning	Afternoon		Morning	Afternoon		
FEV_1 (L)	3.55	3.57	0.70	3.55	3.51	0.32	0.13
FVC (L)	4.49	4.48	0.88	4.46	4.43	0.64	0.83
FEV_1/FVC	0.79	0.80	0.32	0.80	0.80	0.34	0.047
PEF (L/s)	9.35	9.16	0.46	9.08	9.19	0.65	0.28
FEF_{25} (L/s)	7.59	7.64	0.74	7.53	7.50	0.85	0.59
FEF_{50} (L/s)	3.94	3.96	0.83	4.23	4.24	0.95	0.89
FEF_{75} (L/s)	1.48	1.43	0.44	1.51	1.46	0.56	0.85
MMEF (L/s)	3.49	3.47	0.83	3.50	3.50	0.96	0.84

Data are derived from mixed model, accounting for missing data and covariance structure. [a] – indicating probability that cross shift changes during the two seasons differs. FEV – one-second forced expiratory volume. FVC – forced vital capacity. PEF – peak expiratory flow. FEF – forced expiratory flow (at 25, 50, or 75% of vital capacity). MMEF – maximum mid-expiratory flow.

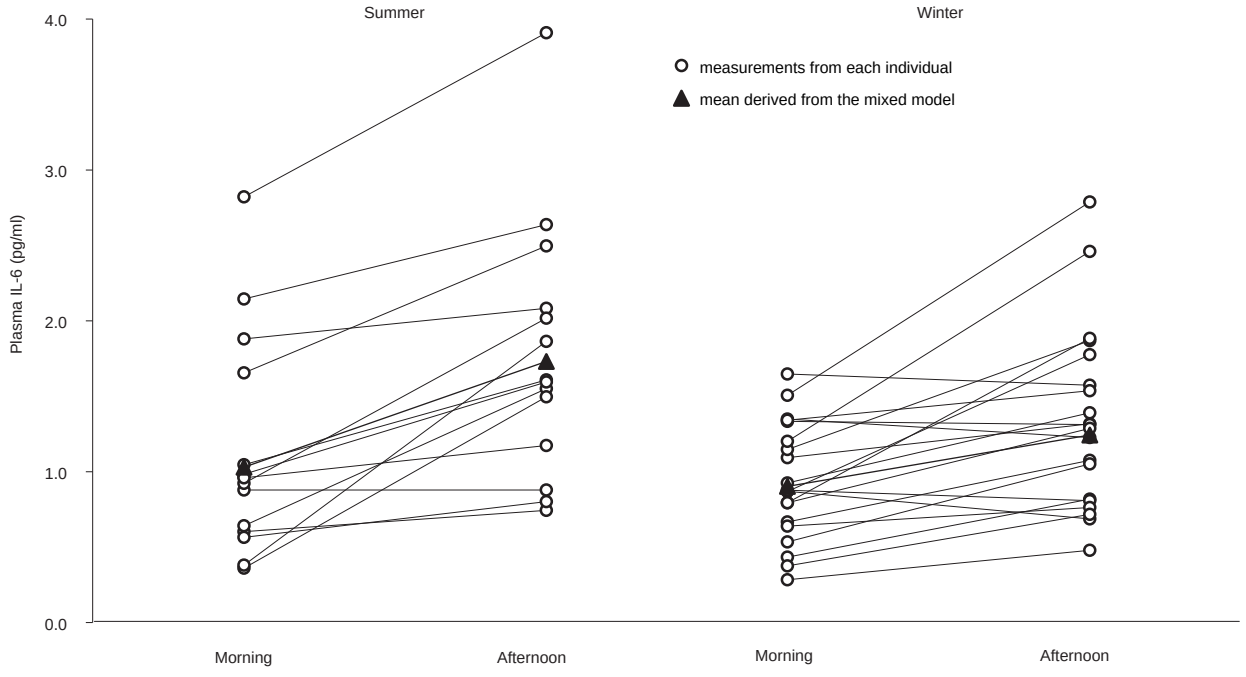

Figure 4. Cross shift changes in plasma IL-6 concentration during summer and winter in the 24 visited workers.

in leukocyte counts, albeit highest during winter. This also supports that the workers adapt but still experience effects during heavy exposures. The seasonality in leukocyte increase could not be ascribed to the neutrophils as, surprisingly, these appeared to increase to the same extent during both summer and winter. No changes in eosinophils were observed, confirming the concept that swine farmers' reactions to their work environment are usually non-allergic. When restricting to the most heavily exposed in the sensitivity analysis, the summer versus winter difference in leukocyte changes tended to wane. Still, even the most heavily exposed during summer reacted less than naïve subjects [2, 3, 24, 25] – supporting that adaptation was not lost during the low exposure summer months.

Indirectly, the observed lack of a significant cross shift decrease in PEF among controls and the tendency for less pronounced changes in FEV_1, FEF_{25} and in blood leukocytes, support that workers experienced inflammatory reactions in the airways at work. The differences are hardly explained by the difference in height between the two groups, as changes over the work shift were compared rather than absolute levels. Any difference in timing of the tests between the two groups is potentially of greater importance because both lung function and leukocyte counts show diurnal variation. The workers spent 414 min (mean), which was 51 min more than the controls, from the morning to the afternoon evaluation. We believe that this difference in timing was too small to explain the opposite patterns in reaction between controls and workers.

Table 4. Blood and plasma markers of inflammation before and after workshift during the two seasons in swine farmers.

	Summer visit		P-value	Winter visit		P-value	P (interaction)[a]
	Morning	Afternoon		Morning	Afternoon		
Blood leucocyte count (*10^9/L)	5.80	6.38	0.014	5.74	6.82	< 0.0001	0.037
Blood neutrophil count (*10^9/L)	3.27	4.09	0.0002	3.38	4.37	< 0.0001	0.43
Blood lymphocyte count (*10^9/L)	1.85	1.96	0.59	1.76	1.85	0.28	0.94
Blood monocyte count (*10^9/L)	0.504	0.538	0.48	0.494	0.497	0.91	0.52
Blood eosinophil count (*10^9/L)	0.155	0.150	0.86	0.161	0.154	0.63	0.98
Blood basophil count (*10^9/L)	0.027	0.029	0.54	0.021	0.032	0.007	0.025
Plasma TNF (pg/ml)	1.34	1.36	0.87	1.34	1.24	0.033	0.014
Plasma TNF-receptor 2 (pg/ml)	1593	1538	0.29	1640	1481	0.003	0.06
Plasma CD62L (ng/ml)	782	808	0.17	761	766	0.78	0.34
Plasma IL-6 (pg/ml)	1.03	1.73	0.0006	0.9	1.24	0.0006	0.14

Data are derived from mixed model, accounting for missing data and covariance structure. [a] – indicating probability that cross shift changes during the two seasons differs.

Table 5. Cross shift changes in lung function and blood leukocytes during winter among workers and controls in the study.

	Workers		Controls		P-value
Cross-shift change	Mean	SD	Mean	SD	
FEV_1 (mL)	-90.5	(113.7)	5.6	(153.2)	0.67
FVC (mL)	-134.3	(167.2)	-5.6	(140.5)	0.53
PEF (mL/s)	-161.9	(510.4)	421.1	(405.9)	0.005
FEF_{25} (mL/s)	-288.6	(506.5)	131.1	(508.6)	0.047
FEF_{50} (mL/s)	-55.7	(514.0)	-136.7	(376.5)	0.67
FEF_{75} (mL/s)	-44.3	(324.3)	-94.4	(188.9)	0.67
MMEF (mL/s)	-41.9	(305.8)	-7.8	(247.0)	0.77
Blood leucocyte count (*10^9/L)	1.624	(0.844)	0.989	(1.052)	0.063
Blood neutrophil count (*10^9/L)	1.395	(0.676)	0.944	(1.008)	0.18

FEV – one-second forced expiratory volume. FVC – forced vital capacity. PEF – peak expiratory flow. FEF – forced expiratory flow (at 25, 50, or 75% of vital capacity). MMEF – maximum mid-expiratory flow.

In studies of effects of work shift exposures it is pertinent to consider the normal diurnal increases in lung function and systemic inflammatory markers from morning to evening. This has been neglected in many previous studies on SCB exposures. Only the deviation from this circadian rhythm can be ascribed to work exposures. Therefore, variation in timing of examinations can contribute to significant variation in results. We aimed at performing the morning examinations at the same time of day, and succeeded in performing the 2nd examination after the same length of exposure during both visits – thus minimizing the variation caused by timing.

Previous research has reported stronger effects on lung function in more experienced workers than in less experienced ones [8, 20]. Years of experience or previous exposures were not controlled for because all workers were compared with themselves. If experienced workers showed stronger responses, they would do so at both visits, still enabling a study of differences in this response between the two visits. The observation of a cross shift decrease in TNF during winter and no change during summer is novel. The majority of previous publications report varying degrees of increase in circulating TNF concentrations after exposure of naïve subjects to swine buildings [3, 24, 25], although some found no changes [2, 18]. Effects on TNF in regularly exposed workers have not been studied, but an experimental re-exposure of former swine farm workers caused a long-lasting depression in plasma TNF [14]. Unless TNF is liberated from inflamed tissue, the plasma levels are probably extremely low or not present at all. Therefore, despite our finding of levels close to the detection limit, the TNF may well be a sign of a chronic inflammatory state due to the work exposure. We cannot rule out the likely explanation that the plasma TNF reached a higher peak shortly after the start of the exposure and was on a decrease from that peak six hours after the start. The analysis was extended with TNF-RII and observed largely the same kinetic as for TNF, corroborating the stronger effect of work during winter than during summer on this part of the immune system. Studies that could confirm the finding of cross-shift downregulation of TNF and its receptor during daily exposure to bioaerosols are warranted.

IL-6, a cytokine that normally comes into play only a few hours after the beginning of acute organic dust exposures, behaved differently than TNF. Plasma IL-6 was clearly more elevated at the end of the work shift. However, its concentration increased less than that which has been observed in naïve volunteers, and the reaction was weaker during winter – maybe as a result of a downregulated TNF response. This may be an important mechanism in adapting to the work environment which could very well be better in the high exposure winter season.

Our finding of little effect on the lungs is in line with some previous studies on low grade exposures [7, 13]. However, during winter we found extremely elevated personally sampled concentrations of endotoxin in the air and levels of total dust and gases comparable to many previous studies (as reviewed by Omland [19]). As previously reported by our group, winter levels of endotoxin and CO_2 were higher and temperature lower than during summer, with RH remaining relatively constant [11]. In that study, the NH_3 concentrations during winter were higher than during summer, and twice as high as in the present study where no seasonal differences were observed. In the present study, dust levels were in the same range during both seasons as in the previous study. The present study showed summer levels of airborne endotoxin in the same range as in the previous study, but winter levels that were much higher. As discussed in [11] this is probably due to methodological differences and reflects a constant strive in our laboratory to improve sampling and analysis of endotoxin in work environments. We believe that the endotoxin concentrations in the $2 * 10^4 – 7 * 10^5$ EU/m^3 range reported here are closer to the true exposures encountered by modern swine workers than the lower exposures in most previous studies. The study was not designed to address which of the airborne components could be responsible for the observed effects.

Despite many efforts, we were not able to include the intended 30 workers. Moreover, those who were included did not always work as long as expected, or could not be visited as many times as intended. We thus ended up with fewer workers and some visits that were not strictly to the protocol, e.g. because of short work days, entry into animal houses before the first evaluation of the day, or little time spent on work with swine. Despite these constraints, a sensitivity analysis could be performed by excluding those with the shortest work exposures. This, and analyses on other subsets of workers (excluding data from days when workers had entered animal houses before the evaluations in the morning; excluding winter visits after 15 March and summer visits before 1 June; or excluding days spent only in farrowing units – data not shown) revealed that the observed differences between effects of working during summer compared with winter were robust. In fact, tendencies of differences in responses between summer and winter in lung function became clearer in these sensitivity analyses. This indicates that variations in exposure not related to season and in timing of visits introduced some noise in the study.

The major strength was the use of the repeated measurements on the same workers in the same work environment in combination with personal assessment of the dust and endotoxin exposures by personal sampling during both visits. The use of mixed models allowed for use of subjects or visits with some missing data. The study of both local respiratory effects and systemic effects allowed us to compare and confirm the direction of the effects caused by the work exposures.

The most important limitation of the study was the small number of persons; that these did not always stick to the protocol and that we had very little control of what they were actually exposed to during visits. Farming is an extremely varied job and it is inherently difficult to standardize exposures in order to improve validity of the observations. The lack of a visit during an unexposed vacation period rendered interpretation of cross-shift changes during the low exposure summer period difficult. Only two blood samples were performed during each visit, and may therefore have missed peaks in inflammatory markers occuring at other times. The 6–7 h of lag from start of work to 2nd blood sample compares well with many other studies and is considered relevant for studies of IL-6 but may not be relevant for TNF, TNF-receptors or CD62L. Finally, the common problem in workplace studies of delays in laboratory analysis caused by the distance to the farms might be of concern. These limitations would tend to induce random noise and complicate finding of true differences related to exposure. The number of statistical tests was high, thus increasing the likelihood of chance findings, and the results should be interpreted with caution.

It is likely that those who choose to participate were not representative of the swine farmers in the region, or compared with other regions in the world. This fact limits the external validity. The exposure levels and the size of the cross shift reactions can only be indicative of reactions in other swine farmers.

Some but not all published studies on swine workers' work shift reactions report on the season and the time of day of investigations. The findings of differences in exposure and in adverse health effects with season in conjunction with the known circadian rhythms lead us to suggest that timing should be considered and reported in such studies. If neglected, comparisons with other studies of the same environment are hampered and the risk of misinterpretation of results increased.

CONCLUSIONS

This study confirmed that there were more moderate negative effects on lung function and the immune system during summer than during winter in swine farm workers in Québec. The hypothesis of limited health effects in exposed workers compared with those reported in the literature on subjects not exposed daily was confirmed. We observed an unexpected decrease in plasma TNF and TNF-RII concentrations over the work shift in winter, which we speculate may be part of the adaptation of the immune system. This study also confirmed that in a climate with cold winters and warm summers, the exposures to endotoxin differed with season and were extremely high during winter.

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