

Work-Related Health Effects in Swine Building Workers After Respiratory Protection Use

Jakob Hjort Bønløkke, MD, Marc Veillette, Anne Mériaux, Caroline Duchaine, PhD, and Yvon Cormier, MD

Objective: To compare inflammation and lung function in swine workers after periods with and without respiratory protection during work. **Methods:** Twenty-three workers were examined before and after two non-protected work shifts. One shift was preceded by a period with diminished exposure by use of respirators. The other shift was preceded by an unprotected period of work. **Results:** Endotoxin concentrations were similarly high (24,636 and 28,775 endotoxin units/m³). A 3.1% cross-shift decline in forced vital capacity occurred after the period with respiratory protection ($P = 0.01$). Blood leukocytes increased more ($P = 0.01$) and bactericidal/permeability-increasing protein was reduced ($P = 0.015$) only after the period with respiratory protection. Plasma interleukin-6 increased ($P < 0.0001$) during both visits. **Conclusion:** Respiratory protection resulted in cross-shift inflammatory and respiratory reactions at return to unprotected work.

Since the early 1980s numerous studies have demonstrated lung function decreases among swine farm operators.¹ Despite these studies and the research on similar exposures in chicken breeding and other farming and nonfarming environments, the determinants of the adverse health effects remain incompletely understood.

In several studies, subjects naive to swine farming have been shown to react strongly with flu-like symptoms and lung function decrements after a few hours of exposure to the swine building environment.¹⁻³ Naive volunteers also benefit substantially from respiratory protection when exposed to such a work environment.⁴ Among daily-exposed swine farm workers, the cross-shift changes in lung function and inflammatory responses are less pronounced than in naive subjects. In previous studies, forced expiratory volume in 1 second (FEV₁) varied from no change⁵ to declines of 3% to 4%.^{1,6,7} Declines in FEV₁ of 6% to 7% were observed in a German study of work compensation claimants with respiratory symptoms.⁸ In that study, half of the subjects abstained from the use of otherwise regularly used respiratory protection.

Although few studies have directly compared reactions in naive subjects with those of workers in regular contact with swine, the emerging picture from the literature is that reactions in the latter are less pronounced.⁹ Furthermore, in our experience it is not uncommon for swine farm workers to report some increase in symptoms upon a return from holidays. The symptoms, which tend to wane after a few days' regular work with swine, encompass flu-like symptoms.

Indeed, severe inflammation has been documented after swine house exposure with release of interleukin (IL)-6 and of granulocytes into bronchoalveolar lavage and blood.^{10,11} Increases in tumor necrosis factor (TNF) have been found in some⁴ but not in all studies,¹² but downstream effects of TNF release probably also depend on the concentration of its receptors. The TNF receptors have been shown to increase locally following bronchial endotoxin instillation in humans.¹³ In that study, no systemic changes in TNF or TNF receptors were reported in contrast to C-reactive protein (CRP), which increased 8 hours after exposure.

Agents in the work environment of swine workers for which an association with the observed health reactions have been observed include dust, endotoxin, and ammonia.^{14,15} Concentrations of these agents are higher inside swine houses during winter.¹⁶ It has been proposed that adaptation or tolerance to endotoxin or other substances in this environment is induced in regularly exposed workers—a phenomenon that is well known among cotton workers heavily exposed to endotoxin-rich dust.^{11,17} Extracellular proteins such as lipopolysaccharide-binding protein (LBP), bactericidal/permeability-increasing (BPI) protein, and soluble cluster of differentiation molecule 14 (CD14) are known to be important in the response to endotoxin because they bind to it and greatly influence the signal produced in response to endotoxin in the organism.¹⁸ The balance between membrane-bound and soluble protein is important in determining the function of several inflammatory mediators. In addition to TNF receptors and CD14, this is true also for soluble L-selectin (called CD62L), an adhesion molecule, for which enhanced serum concentrations in swine farm workers have previously been observed, possibly due to increased shedding from cell membranes.¹⁹

The aim of this study was to verify whether the adaptation of swine farm workers would wane or even be lost after a short period of respiratory protection. We hypothesized that (1) daily exposure to swine confinement buildings activates adaptive mechanisms and results in diminished inflammatory responses; and (2) this adaptation to the work environment is lost, or at least decreased, after 4 days of minimization of exposures by the use of efficient respiratory protection.

MATERIALS AND METHODS

Study Subjects and Design

Swine farm workers (operators and owners) within 150 km of Quebec City were visited twice during winter (between October 18 and April 26 in both 2005 and 2006). All farms visited had at least one mechanically ventilated finishing building. Smokers and workers reporting respiratory infections during the 7 days preceding the visits or lung diseases were excluded. Health examinations took place in the morning immediately prior to the first entry of the worker into the animal house and at the end of the work shift. More detailed information has been published elsewhere.²⁰

At one of the two visits, the workers were examined after an ordinary unprotected period of work on the swine farm of at least 4 days. Before the other of the two visits, the workers were requested to abstain from exposure to swine and swine houses for at least 4 days. In cases where this could not be achieved, they were requested to wear N95 respirators (3M, St Paul, MN) during all entries into

[AQ1] From the Department of Environmental and Occupational Health (Dr Bønløkke), Institute of Public Health, Aarhus University, Aarhus, Denmark; and Unité de Recherche (Mr Veillette, Ms Mériaux, and Drs Duchaine and Cormier), centre de Pneumologie, Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ), Université Laval, Québec, Canada.

[AQ2] The study was funded by the Fonds québécois de la recherche sur la nature et les technologies in collaboration with the Institut de recherche Robert-Sauvé en santé et sécurité du Travail. Jakob Bønløkke was a CIHR Strategic Training Fellow in PHARE, research funded by the CIHR Strategic Training Program and Partner Institutes.

All of the authors declare that they have no conflicts of interest related to this study.

Address correspondence to: Jakob Bønløkke, MD, Department of Environmental and Occupational Health, Institute of Public Health, Aarhus University, Bartholins Allé 2, DK-8000 Aarhus C, Denmark (jb@mil.au.dk).

Copyright © 2012 by American College of Occupational and Environmental Medicine

DOI: 10.1097/JOM.0b013e31825461f4

swine houses for protection of the airways. These visits are referred to as visits after respirator use. Correct use of the respirators was demonstrated and the fit checked by one of the investigators during a visit to the farm.

Complete spoken and written information about the study was given prior to the first evaluation and an informed consent to participate was obtained. The study was approved by the institutional ethics committee at the Unité de Recherche, Centre de Pneumologie, Institut Universitaire de Cardiologie et de Pneumologie de Québec.

Exposure Assessments

On visit days, the workers were equipped with four personal filter samplers attached to pumps and asked to carry these from entry into the swine houses until end of work within these houses. Control filters were brought to the sampling site and exposed, but not subjected to sampling, and were assessed by the same procedures as filters subjected to sampling. Upon return to the laboratory, endotoxin measurements were performed in duplicate for each filter using state-of-the-art methods as previously described.¹⁶ Dust concentrations were calculated by gravimetric measurements. During each visit, one building with hogs was selected for stationary continuous sampling of temperature, relative humidity, CO₂, and NH₃ for at least 120 minutes with calibrated devices. Details about the exposure assessments have been published elsewhere.²⁰

Health Examinations

During the first visit, a respiratory health history was taken for each subject on the basis of questions derived from the standard American Thoracic Society questionnaire for respiratory diseases²¹ with questions about current and past jobs, use of respiratory protection, dust, and gas exposures added. Height and weight were measured. At the second visit, questions about respiratory protection, medication, and work in the previous 7 days were repeated. All workers performed spirometry before and after work following American Thoracic Society guidelines²² with a 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, as described elsewhere.²⁰ Equations for predicted volumes were taken from Quanjer et al²³ Forced vital capacity (FVC), FEV₁, peak expiratory flow, forced expiratory flow (FEF) at 25%, 50%, 75% of expired volume, and maximal mid-expiratory flow were recorded.

Exhaled Breath Condensate

Exhaled breath condensate (EBC) was collected in iced glass cylinders developed for this study. Before visits, isolated boxes were filled with ice packs and ice around a cleaned, sterilized, and sealed vertical cylinder (Pyrex 100-mL glass cylinder). These boxes were kept frozen until use. Disinfected tubes, valves, and mouthpieces (Hudson RCI Unidirectional Valved Tee, catalogue 1666; Hudson Respiratory Care Inc, Temecula, CA) were assembled and mounted (after removal of the sealing foil) on the cylinder. The worker was instructed to breathe normally or slightly deeper than normal into the mouthpiece after swallowing excess saliva. Exhaled air was directed to the glass cylinder via a T-piece with one-way valves. Inspiration was either through the T-piece or by nasal inspiration. A nose clip was not used. The worker was encouraged to continue for 10 minutes. Excess saliva during this time could be swallowed or expelled. After 10 minutes, the flexible tube was removed, except for the “out” valve, which was kept connected to the interior tube of the cylinder. This partly sealed off the cylinder. Care was taken to avoid introduction of saliva from the removed tubes to the cylinder.

When thawed at room temperature, the EBC was transferred to 1.5-mL sterile polypropylene tubes and stored on dry ice until returned to the laboratory, where they were stored at -70°C . Exhaled breath condensate was obtained both before and after the work shift.

Condensates were entered into an anaerobic chamber (Thermos Forma Anaerobic System model 1025/1029; Thermos Forma, Marietta, OH) in an atmosphere of 100% nitrogen. The level of CO₂ was monitored with a Q Trak Plus model 8552 (TSI Inc, Shoreview, MN) to assure concentration close to zero. The EBC pH was measured with an Accumet Basic AB 15 pH meter (Fisher Scientific). After at least 1 hour in this atmosphere, pH measurements were initiated. The IL-8 concentration was measured with a chemiluminescent enzyme-linked immunosorbent assay (ELISA) (QuantiGlo, R&D Systems, Minneapolis, MN) on thawed EBC without conditioning the atmosphere.

Blood Samples

Venous blood samples were taken before and after work at each visit in standard tripotassium ethylenediaminetetraacetic acid-coated tubes. One tube was kept at room temperature for white blood cell count. At the end of each clinical evaluation the remaining tubes were centrifuged 10 minutes at 1200 $\times g$. The plasma was then frozen on dry ice. After removal of the plasma, approximately 500 μL of the white blood cell layer (Buffy coat) was mixed with 4 mL Trizol Reagent (Invitrogen Corporation, Burlington, Ontario, Canada). After mixing, 1-mL aliquots were added to polypropylene tubes and placed on dry ice. Upon return to the laboratory after the final examination of the day, white blood cell counts were performed and the remaining samples stored at -80°C for later analyses. CRP was measured by immunonephelometry with a high-sensitivity CRP reagent on a BNProSpec (Dade Behring, Marburg, Germany). The concentrations of TNF, TNF-receptor Type A (TNF RII), IL-6, CD14, and CD62L were measured with ELISA kits from R&D Systems. BPI protein and LBP were measured with ELISA kits from Hycult (Hycult Biotechnology, Uden, the Netherlands). According to the manufacturers, 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, 0.28 pg/mL for IL-8, 125 pg/mL for CD14, 0.3 ng/mL for CD62L, and 250 pg/mL for BPI protein. No limit of detection was provided for LBP.

Statistical Methods

Means were compared using either Wilcoxon signed rank or *t* test, depending on distributions. Repeated measures were analyzed using mixed models considering subjects 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 visits and the other fixed factor to the time (morning vs afternoon). Because subjects were visited twice, there were repeated measurements for subjects to identify the different responses at the different visits. For this repeated factor, an unstructured (UN) covariance structure was defined to take into account the dependency among visits. At each visit we obtained measurements in the morning and by the end of the day. This second factor is another repeated factor to identify the different measurement times within visit. Thus, within each UN structure, an autoregressive covariance structure is defined to take into account the dependency between measurements within the visit. Thus, when combined, an UN@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 environmental exposure variables were included as covariates in the repeated measures analyses after transformations approximating them to the normal distribution. *P* values of 0.05 or less were considered statistically significant. The repeated measures analyses were conducted with SAS v9.1.3 software (SAS Institute, Cary, NC). SPSS v13.0 (SPSS, Inc, Chicago,

[AQ3]

IL) was used for the remaining analyses. Sensitivity analyses were performed by the same methods after exclusion of the least-exposed subjects as described in more detail elsewhere.²⁰

RESULTS

A total of 23 workers (2 women and 21 men) were included in the study and visited twice during winter. Their characteristics, including reported use of ordinarily used respiratory protection and reported symptoms when off work, are listed in Table 1. For at least 4 days prior to the visits after respirator use, workers were either protected by wearing a respirator only ($n = 14$), by complete avoidance of the swine buildings ($n = 1$), or by a combination of the two ($n = 6$). Information is missing for two workers. The work conditions and exposures at the visits are provided in Table 2. None of the environment variables were found to differ between the two visits.

The primary outcomes of interest are listed in Table 3 and in Fig. 1. FVC decreased 3.1% over the work shift after the period of respirator use whereas it showed no cross-shift decline after the unprotected work period (Fig. 1). This interaction between visit and time was statistically significant ($P = 0.01$).

Peak expiratory flow, FEV₁, FEV₁/FVC, FEF₂₅, FEF₅₀, FEF₇₅, and maximal mid-expiratory flow did not show any cross-shift changes at either of the two visits, although there was a tendency for a cross-shift decrease after the respirator use in FEV₁ ($P = 0.07$). No statistically significant changes in the pH of the EBC appeared with visit or across the work shift, although it tended to be higher ($P = 0.1$) at days after work with respiratory protection. In the EBC samples IL-8 was below the detection limit of the assay.

The blood leukocyte concentration increased over the work shifts ($P < 0.0001$ at both visits) (Fig. 1 and Table 3). After the respirator use period the increase in leukocytes was 28.2% compared with an increase of 18.6% after the unprotected work period ($P = 0.01$ for an interaction between visit and time). The cross-shift increases in leukocyte concentrations mostly reflected an increase in neutrophils ($P < 0.0001$ at both visits) (Fig. 1). The neutrophil fraction of the leukocyte count increased across the work shift to the same extent at both visits without indication of differences between visits.

An interaction between visit and time appeared, indicating a greater cross-shift decline in the eosinophil fraction of leukocytes after the respirator use than after ordinary work periods ($P = 0.02$)

TABLE 1. Characteristics of the Study Population

Characteristic	N = 23
Age, mean (range), yr	41.5 (20–69)
Height, mean (range), cm	171.9 (157–186)
Weight, mean (range), kg	79.1 (54–123)
Body mass index, mean (range)	26.6 (20.9–38.8)
FVC in % of predicted, mean (range)	103.6 (81.2–127.3)
FEV1 in % of predicted, mean (range)	100.4 (76.9–127.9)
Years working with swine, mean (range)	16.4 (1–50)
Work hours on swine farm/week, mean (range)	41.0 (28–60)
Some use of respiratory protection, n (%)	5 (21.7)
Improved respiration when not at work, n (%)	9 (50% of 18 responders)

TABLE 2. Work Conditions and Exposures During the Two Study Visits

Conditions	Study Visit	
	After ≥4 Days of Respirator Use	After ≥4 Days of Unprotected Work
Duration from work start to second clinical evaluation, mean (SD), min	369 (108)	372 (68)
Duration of work with swine, mean (SD), min	259 (96)	231 (109)
Personal dust exposure, mean (SD), mg/m ³	4.33 (3.08)	3.86 (1.99)
Personal endotoxin exposure, mean (SD), EU/m ³	24636 (17580)	28775 (17312)
Area per animal, mean (SD), m ²	1.23 (0.48)	1.1 (0.41)
Indoor temperature, mean (SD), °C	18.4 (2.1)	19.3 (1.9)
Relative humidity, mean (SD), %	68.6 (10.3)	66.6 (13.9)
CO ₂ , mean (SD), ppm	2753 (638)	2671 (882)
NH ₃ , mean (SD), ppm	17.5 (14.8)	17.9 (15.1)

EU, endotoxin units; SD, standard deviation.

(Fig. 1). In contrast, the counts of lymphocytes and basophiles increased over the work shift in both work situations ($P < 0.05$ after respirator use and $P = 0.001$ after unprotected work—data not shown). Neither of these two subpopulations of leukocytes demonstrated any differences between visits.

As shown in Table 3, a tendency for plasma TNF concentrations to decrease over the work shift at both visits was observed ($P < 0.06$). For plasma TNF RII, a statistically significant decrease with time across the work shift was observed at both visits ($P < 0.0001$) without signs of different patterns between visits. After the respirator use the change was -10.8% and after the unprotected work it was -9.2% (Fig. 1).

In contrast to TNF, the plasma IL-6 concentration increased over the work shifts ($P < 0.0001$) (Table 3). After the respirator use period the increase was 69.5% and after the unprotected work period 35.0% (Fig. 1). This difference in increase was not statistically significant ($P = 0.06$).

The plasma CD62L concentrations did not change across either of the work shifts. Nonetheless, we observed an interaction between visit and time ($P < 0.03$), revealing that CD62L tended to diminish from slightly elevated concentrations after the respirator use period (-2.2%) compared with a tendency to rise from slightly lower concentrations after the unprotected exposure period ($+1.4\%$).

After the respirator use period the plasma concentration of BPI protein declined (-63% ; $P = 0.007$) over the work shift whereas it did not decline after the unprotected work ($P = 0.6$) (Fig. 1). This interaction between visit and time was statistically significant ($P = 0.015$). The concentrations of LBP, CD14, and CRP were not influenced by the work shift or by visit.

It was observed that in the mornings before the beginning of the day's work the majority of the investigated outcomes did not differ between the two visits despite different exposures in the 4 or more preceding days. Only plasma BPI protein was higher in

TABLE 3. Blood and Plasma Markers of Inflammation Before and After the Work Shift During the Two Visits

	After ≥4 Days of Respirator Use, Mean (SE)		P	After ≥4 Days of Unprotected Work, Mean (SE)		P	P _{interaction} *
	Morning	Afternoon		Morning	Afternoon		
Blood leucocytes (×10 ⁹ /L)	5.56 (1.56)	7.13 (1.56)	0.0001	5.80 (1.04)	6.88 (1.04)	0.0001	0.0101
TNF, pg/mL	1.18 (1.08)	1.13 (1.08)	0.15	1.32 (1.08)	1.23 (1.08)	0.07	0.7495
TNF RII, pg/mL	1659 (1.05)	1479 (1.05)	0.0001	1618 (1.05)	1469 (1.05)	0.0007	0.5552
IL-6, pg/mL	1.05 (1.13)	1.78 (1.13)	0.0001	1.00 (1.10)	1.35 (1.10)	0.0021	0.0599
CD62L, ng/mL	803 (23)	785 (23)	0.17	768 (28)	779 (28)	0.81	0.0267
CD14, ng/mL	1.46 (0.03)	1.50 (0.03)	0.11	1.57 (0.04)	1.55 (0.04)	0.67	0.0883
CRP, μg/mL	1.00 (0.18)	0.99 (0.18)	0.91	1.04 (0.15)	1.00 (0.15)	0.0509	0.1246
BPI, ng/mL	47.8 (1.30)	17.7 (1.29)	0.0072	25.6 (1.30)	21.5 (1.29)	0.61	0.0151
LBP, ng/mL	111.7 (1.05)	107.3 (1.05)	0.25	116.1 (1.07)	120.0 (1.07)	0.49	0.1137

Data are derived from the mixed model, accounting for missing data and covariance structure. BPI, bactericidal/permeability-increasing protein; CD14, soluble cluster of differentiation molecule 14; CD62L, soluble L-selectin; CRP, C-reactive protein; IL-6, interleukin-6; LBP, lipopolysaccharide-binding protein; SE, standard error; TNF, tumor necrosis factor; TNF RII, TNF-receptor Type A.

*Indicating the probability that the changes over the work shift differ between the two visits.

the morning after the respirator use period than it was after the unprotected exposure period ($P < 0.02$).

Restriction of the analyses to the subset of workers and visits for which complete environmental data were available (varying from 2.2% missing data in the case of endotoxin and dust to 32.6% missing data in the case of CO₂) resulted in apparent effects of time and visit on several of the outcome variables (data not shown). In all cases these effects on the outcomes were related to the restriction of the population caused by missing data from days when environmental sampling had failed and they could not be shown to relate to the environment. Of the environmental variables, only the CO₂ concentration was associated in a statistically significant way with more than one outcome. CO₂ was associated with FEV₁, FEV₁/FVC, and CD62L (P values between 0.01 and 0.03). For all three outcomes, greater cross-shift decreases with higher CO₂ concentrations were observed.

Restricting the analyses to the 14 subjects (2 women and 12 men), which adhered strictly to the respirator carriage protocol, caused the statistical significance of the greater FVC decline after the respirator use period to disappear, although the pattern remained. The greater cross-shift decline in the eosinophil fraction of leukocytes after the respirator use period than after the ordinary work period also became insignificant. In this restricted population, a pattern of decline in FEF₅₀ during the workday after the respirator use period as opposed to an increase after the ordinary work period reached statistical significance ($P = 0.01$). Furthermore, increases appeared in the neutrophil fraction of the leukocyte count ($P = 0.03$), plasma CD14 ($P = 0.05$), and CRP ($P = 0.02$) that were greatest on the workdays after the respirator use period than on the workdays after the ordinary work period. The lymphocyte fraction of the leukocyte count was higher after the ordinary work period than after the respirator use period ($P = 0.04$) but the cross-shift increase remained during both visits ($P = 0.05$). Declines in plasma BPI protein occurred at both visits ($P = 0.005$) but remained more pronounced after the respirator use period ($P = 0.02$). Lower leukocyte ($P = 0.04$) and lymphocyte ($P = 0.02$) counts were observed in the mornings after the respirator use period than after the ordinary work period. The elevated plasma BPI protein in the morning after the respirator use period compared with that after the ordinary work period remained ($P = 0.0009$).

Restriction of the analyses to the subjects exposed to work inside buildings with swine for at least 3 hours on the day of the visit confirmed the greater increase in blood leukocytes after the

respirator use period ($P = 0.0004$) as well as the decrease in plasma TNF RII ($P = 0.0035$) and increase in plasma IL-6 concentrations ($P = 0.0124$) during both visits. The cross-shift decline in FVC after the respirator use period and the differential plasma BPI protein changes disappeared with this restriction. However, these differences in cross-shift changes of FVC and BPI protein remained when restricting the analysis only to subjects that were primarily exposed during the first half of the workday ($P = 0.0088$ and 0.0487 , respectively).

DISCUSSION

We observed differences in cross-shift change in FVC and immune reactions in swine farm workers despite similar work conditions at the two visits. By design, the workers had experienced different exposures in the days prior to their examinations by having been off work or carrying respiratory protection for at least 4 full days before one but not the other of the two visits. The findings support our hypothesis of some loss of adaptation during time off work that causes greater effects on respiratory function and the immune system when returning to work.

Such a pattern of greater adverse health effects at return to work is known from other occupations; byssinosis in cotton workers has been studied most.²⁴ Endotoxins have been identified as the agent primarily responsible for that condition.²⁵ Experimental studies have demonstrated lung function decrements, blood neutrophilia, and increasing plasmatic levels of inflammatory mediators (such as TNF and IL-6) in response to inhalation of endotoxin or endotoxin-rich organic dust such as dust from swine farms, as reviewed by Rylander.²⁶

This study confirms that working in swine confinement buildings results in cross-shift decline in lung function and increases blood neutrophils and IL-6. In contrast to previous studies with subjects naive to the environment^{10,12,27} (where circulating TNF levels either increased or remained constant at high concentrations), plasma TNF stayed at low concentrations and tended to decrease further during both visits. This could be an indication of a difference in the immune reactions between a first time exposure and repeated exposures. A pathway not involving TNF could be responsible for the cellular reactions in regularly exposed swine farm workers. Alternatively, because of a state of alertness, an earlier and smaller TNF response may have been present in the regularly exposed workers. Thus, TNF could have returned to normal levels by the time of the blood sam-

[AQ4]

[AQ5]

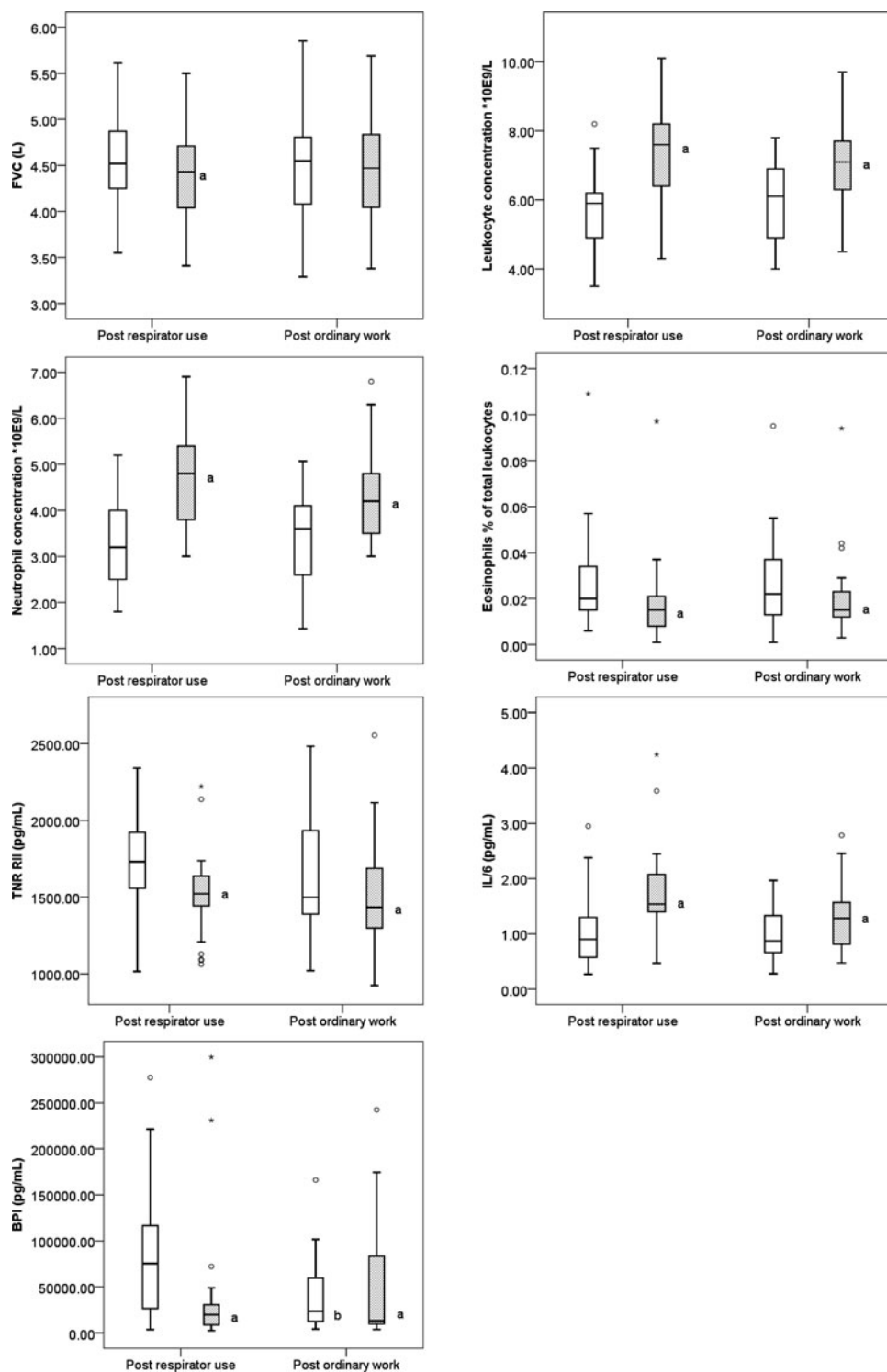


FIGURE 1. Health outcomes measured before work in the swine building (white) and in the afternoon at the end of the work shift (gray) during visits after a period of respiratory protection use and after a period with ordinary work conditions.

pling after the work shift. Four days of respirator use was not enough to change the pattern and cause the TNF burst normally associated with endotoxin exposure. The IL-6 response, which has been shown to be partly independent of the TNF response to organic dust,²⁸ was conserved despite the lack of observed TNF increase.

This study included cross-shift changes in markers of inflammation that have not previously been applied to studies of swine farm workers. We observed a cross-shift decline in TNF RII and a tendency for a BPI protein decline, whereas no changes in CRP were observed. When restricting the analysis to the workers adhering

strictly to the protocol of 4 days of respirator use, greater plasma BPI protein declines and greater CD14 and CRP increases were observed on the work day after respirator use than after ordinary work.

Such an interaction between time and visit was statistically significant for CD62L as well. For the latter, opposite patterns were observed at the two visits with tendencies for cross-shift increases in concentrations after the ordinary work period and declines after the days with use of respiratory protection. CD62L concentrations were comparable with those of unexposed controls but lower than those in swine farm workers in a study by Israël-Assayag and Cormier.¹⁹ We have no explanation for this, other than differences in batch numbers and possibly differences in timing between sampling and assessment. On the basis of the hypothesis proposed in that article,¹⁹ one would expect higher CD62L concentrations during the ordinary work period, something that we did not observe. A possible explanation could be that CD62L concentrations take longer to change than the 4 or more days of work exposure prior to its measure in this study. This would be in line with the finding that the concentration of CD62L in sera from volunteers did not change after seven weekly 4-hour exposures to swine confinement buildings.¹⁹ The change in CD62L was not robust to restriction of the analysis to the subset of workers that were most heavily exposed during visits and could be spurious.

When interpreting cross-shift changes it is important to take the normal diurnal variation into account. The normal increase in lung function from early morning to the afternoon was not observed at either visit, indicating a negative effect of the swine building environment. As reported previously, we did observe the expected diurnal increase in healthy controls.²⁰ For the mediators of inflammation, the diurnal variations are less well established. IL-6 has a diurnal rhythm with a nadir in healthy normal sleepers in early morning²⁹ and blood leukocyte concentrations also increase during the day. However, the minor differences between visits in timing of the blood samples are unlikely to be the cause of the observed differences in the kinetics of markers of inflammation.

In addition to the observed differences in cross-shift changes, it seemed that the chronic effects of daily swine work exposure tended to wane when the exposure had been interrupted. It was interesting to note that, although far from statistically significant, all the spirometry indices were in the direction of better lung function in the mornings after 4 or more days of respiratory protection. Also suggestive of less inflammation was the tendency of the pH of EBC to be higher on days after work with respiratory protection. BPI protein is mainly found in granules of neutrophils in contrast to LBP, which is mainly (but not solely) synthesized by the liver. BPI protein has greater affinity for LPS than does LBP and, when binding, inhibits LPS-induced inflammation. In contrast, circulating CD14 is part of a small group of receptors, which, in cooperation with LBP, signals increased inflammation in response to LPS. If this signal is strong enough it is likely to result in greater TNF, IL-6, and CRP levels.

The statistically higher plasma BPI protein concentration observed in the morning after the days off exposure and the greater decline during work compared with the morning and days after ordinary work could be an indication of greater use of BPI protein locally in the lungs by endotoxin-rich particles on ordinary work days. This would inhibit inflammation in an adapted subject. Indeed, less of the inflammatory signals were seen in these subjects; CD14 and CRP decreased across the work-shift in contrast to the days after the period with respiratory protection use where these proteins increased, albeit from lower levels. Our results suggest more of a chronic, slowly resolving inflammatory state in workers with daily SCB exposure and greater responses from a less alert state in workers returning from a period with respiratory protection (or, possibly, SCB avoidance).

The strengths of this study were the repeated measurements of the workers at the same time of day, thus eliminating many potential confounders. The inclusion of environmental exposure assessment

allowed for detailed characterization of the work conditions and for verification that these were similar during the two visits. This similarity was confirmed by detailed analysis of the bacterial load, as reported elsewhere.³⁰ Finally, EBC and plasma was analyzed for mediators of inflammation not previously described in studies of swine farm workers.

A limitation of the study was the lack of a proper absence period from work. For practical reasons, this was impossible to organize and on most days, the use of fitted respiratory protection had to replace days off work. Wearing a simple mask even less efficient than the N95 respirator used in this study is known to be effective in swine building exposures.³¹ For practical reasons, the fit and use of the respirators could be controlled only once per worker. In addition, a few workers used respirators for short-term, specific tasks during the visits, thus minimizing exposures when they were supposed to work fully exposed. Another limitation was the small size of the study. The difficulties mentioned most likely introduced random noise into the data. The number of statistical tests was high, potentially resulting in chance findings. However, because most of the findings were consistent in the same direction or remained when restricting to the workers most heavily exposed, the risk that our conclusions are based on spurious findings is small. A potential source of bias was that the investigator was not blinded to the visit type and may have behaved differently when performing the clinical examinations. However, spirometry is highly effort independent and thus the results should not be influenced by that knowledge, and the laboratory analyses were performed by blinded technicians.

The selection of workers most likely resulted in a group, which may not be comparable with all swine farm workers. Subjects were motivated to participate in the study and smokers were excluded. Therefore, our results cannot be generalized to all SCB workers. From a clinical point of view, our findings of increased responses after a few days of respirator use or time off work may be suggestive of greater negative health effects of interrupted than of regular exposure in modern swine farming. Because, among the subjects of our study, it seems that wearing a mask on an irregular basis may be worse than wearing no mask at all, our recommendation is that workers should always wear one.

In conclusion, this study suggests a diminished adaptation of swine farm workers to their work environment after a few days off work or with use of respiratory protection resulting in greater systemic inflammatory responses during work after such a period than after a period of regular exposure. Loss of adaptation did not seem to be complete, as suggested by the absence of a cross-shift burst in TNF. Thus, the TNF pathway may not be responsible for the effects observed whereas BPI protein showed changes compatible with an adaptive effect during regular exposure to the swine building environment.

ACKNOWLEDGMENT

The authors thank Serge Simard for developing the statistical models and Lynn Atton for developing the EBC collectors.

REFERENCES

1. Donham KJ, Zavala DC, Merchant J. Acute effects of the work environment on pulmonary functions of swine confinement workers. *Am J Ind Med.* 1984;5:367-375.
2. Malmberg P, Larsson K. Acute exposure to swine dust causes bronchial hyperresponsiveness in healthy subjects. *Eur Respir J.* 1993;6:400-404.
3. Larsson KA, Eklund AE, Hansson L-O, Isaksson B-M, Malmberg PO. Swine dust causes intense airway inflammation in healthy subjects. *Am J Respir Crit Care Med.* 1994;150:973-977.
4. Wang Z, Manninen A, Malmberg P, Larsson K. Inhalation of swine-house dust increases the concentrations of interleukin-1 beta (IL-1 beta) and interleukin-1 receptor antagonist (IL-1ra) in peripheral blood. *Respir Med.* 1998;92:1022-1027.
5. Heederik D, van ZR, Brouwer R. Across-shift lung function changes among pig farmers. *Am J Ind Med.* 1990;17:57-58.

[AQ6]

6. Donham KJ, Haglund P, Peterson Y, Rylander R. Environmental and Health Studies in Swine Confinement Buildings. *Am J Ind Med.* 1986;10:289–293.
7. Kirychuk S, Senthilselvan A, Dosman JA, et al. Predictors of longitudinal changes in pulmonary function among swine confinement workers. *Can Respir J.* 1998;5:472–478.
8. Radon K, Garz S, Schottky A, et al. Lung function and work-related exposure in pig farmers with respiratory symptoms. *J Occup Environ Med.* 2000;42:814–820.
9. Palmberg L, Larsson B-M, Malmberg P, Larsson K. Airway responses of healthy farmers and nonfarmers to exposure in a swine confinement building. *Scand J Work Environ Health.* 2002;28:256–263.
10. Wang Z, Malmberg P, Larsson P, Larsson BM, Larsson K. Time course of interleukin-6 and tumor necrosis factor-alpha increase in serum following inhalation of swine dust. *Am J Respir Crit Care Med.* 1996;153:147–152.
11. Cormier Y, Duchaine C, Israël-Assayag E, Bédard G, Laviolette M, Dosman J. Effects of repeated swine building exposures on normal naive subjects. *Eur Respir J.* 1997;10:1516–1522.
12. Cormier Y, Laviolette M, Bedard G, Dosman J, Israel-Assayag E. Effect of route of breathing on response to exposure in a swine confinement building. *Am J Respir Crit Care Med.* 1998;157:1512–1521.
13. O'Grady NP, Preas HL, Pugin J, et al. Local inflammatory responses following bronchial endotoxin instillation in humans. *Am J Respir Crit Care Med.* 2001;163:1591–1598.
14. Reynolds SJ, Donham KJ, Whitten P, Merchant JA, Burmeister LF, Popen Dorf WJ. Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. *Am J Ind Med.* 1996;29:33–40.
15. Donham KJ, Reynolds SJ, Whitten P, Merchant JA, Burmeister L, Popen Dorf WJ. Respiratory dysfunction in swine production facility workers: dose-response relationships of environmental exposures and pulmonary function. *Am J Ind Med.* 1995;27:405–418.
16. Duchaine C, Grimard Y, Cormier Y. Influence of building maintenance, environmental factors, and seasons on airborne contaminants of swine confinement buildings. *AIHAJ.* 2000;61:56–63.
17. Von Essen S, Romberger D. The respiratory inflammatory response to the swine confinement building environment: the adaptation to respiratory exposures in the chronically exposed worker. *J Agric Saf Health.* 2003;9:185–196.
18. Sigsgaard T, Hoffmann HJ, Thorne PS. The role of innate immunity in occupational allergy: recent findings. *Curr Opin Allergy Clin Immunol.* 2008;8:120–125.
19. Israël-Assayag E, Cormier Y. Adaptation to organic dust exposure: a potential role of L-selectin shedding? *Eur Respir J.* 2002;19:833–837.
20. Bønløkke JH, Mériaux A, Duchaine C, Godbout S, Cormier Y. Seasonal variations in work-related health effects in swine farm workers. *Ann Agric Environ Med.* 2009;16:1–10.
21. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis.* 1978;118:1–120.
22. The American Thoracic Society. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med.* 1995;152:1107–1136.
23. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Volumes pulmonaires et débits ventilatoires forcés. Groupe de travail sur la standardisation des épreuves fonctionnelles respiratoires. Communauté européenne du charbon et de l'acier. Position officielle de l'European Respiratory Society. *Rev Mal Respir.* 1994;11(suppl 3):5–40.
24. Lane SR, Nicholls PJ, Sewell RD. The measurement and health impact of endotoxin contamination in organic dusts from multiple sources: focus on the cotton industry. *Inhal Toxicol.* 2004;16:217–229.
25. Castellan RM, Olenchock SA, Hankinson JL, et al. Acute Bronchoconstriction Induced by Cotton Dust: dose-related responses to endotoxin and other dust factors. *Ann Intern Med.* 1984;101:157–163.
26. Rylander R. Endotoxin in the environment—exposure and effects. *J Endotoxin Res.* 2002;8:241–252.
27. Malmberg P, Zhiping W, Larsson P, Larsson K, Isakson B-M. Exposure to swine dust causes IL-6 but not TNF increase in serum *Eur Respir J.* 1993;6(suppl 17):476s.
28. Burvall K, Palmberg L, Larsson K. Expression of TNFalpha and its receptors R1 and R2 in human alveolar epithelial cells exposed to organic dust and the effects of 8-bromo-cAMP and protein kinase A modulation. *Inflamm Res.* 2005;54:281–288.
29. Vgontzas AN, Bixler EO, Lin HM, Prolo P, Trakada G, Chrousos GP. IL-6 and its circadian secretion in humans. *Neuroimmunomodulation.* 2005;12:131–140.
30. Nehme B, Letourneau V, Forster RJ, Veillette M, Duchaine C. Culture-independent approach of the bacterial bioaerosol diversity in the standard swine confinement buildings, and assessment of the seasonal effect. *Environ Microbiol.* 2008;10:665–675.
31. Dosman JA, Senthilselvan A, Kirychuk SP, et al. Positive human health effects of wearing a respirator in a swine barn. *Chest.* 2000;118:852–860.

[AQ7]

Title: Work-Related Health Effects in Swine Building Workers After Respiratory Protection Use
Author: Jakob Hjort Bønløkke, Marc Veillette, Anne Mériaux, Caroline Duchaine, Yvon Cormier

Author Queries

- [AQ1]: Please verify the affiliations and the correspondence address. Also, provide the professional degrees of the authors, Marc Veillette and Anne Mériaux.
- [AQ2]: Please verify the disclosure statement.
- [AQ3]: Please provide the location of “Fisher Scientific.”
- [AQ4]: Please check whether the sentence, “The elevated plasma BPI in the morning . . .” is complete as given.
- [AQ5]: Please verify the changes in the sentence, “Thus, TNF could have returned to normal levels by the time . . .”
- [AQ6]: Please provide the expansion of “SCB.”
- [AQ7]: Please verify the suggested vol. no. in ref. 27.