ORIGINAL RESEARCH

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Respiratory and systemic health parameters in pigs raised in a conventional farm or in isolation

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Summary

Objective: To examine the longterm effects of the farm environment—including exposure to airborne contaminants and disease agents—on animal health by raising pigs from the same farm in the conventional farm environment or in an isolated facility.

Methods: The farm was selected for the high prevalence of pneumonia and active porcine reproductive and respiratory syndrome virus (PRRSV). One-week-old pigs were raised on the farm (Farm), or were weaned at 2 weeks of age and raised in an isolated research facility (ISO). Bronchoalveolar lavage fluid (BALF) was collected at 5, 7.5, 10, 15, and 20 weeks of age for cell differentials, superoxide anion (SO), and phagocytosis activity. Blood samples were analyzed for cell type and numbers, and respiratory disease antibodies. At 20 weeks of age, pigs were slaughtered and respiratory tracts were examined. The airborne environment of farm and isolated housing was sampled for dust, endotoxin, peptidoglycan, ammonia, and carbon dioxide.

Results: Farm pigs seroconverted to PRRSV and *Mycoplasma hyopneumoniae* after 10 weeks of age, which resulted in increased BALF cell infiltration, increased SO, and lower phagocytosis activity compared to ISO pigs. ISO pigs also had activated BALF cells, as suggested by the increased SO and phagocytosis activity. Macroscopic pneumonia lesions, indicative of *M. hyopneumoniae*, were only observed in the Farm pigs. Airborne contamination was greater in the farm environment than in the isolated environment.

Implications: Housing pigs in a clean, isolated, disease-free, and low-stress environment positively influences health of pigs. Minimal airborne contamination is sufficient to induce cellular changes in BALF and infectious agents, such as PRRSV, could further alter the BALF cellular composition and cell activity.

Keywords: swine, environment, dust, endotoxin, respiratory disease, bonchioalveolar lavage

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Respiratory disease in pigs continues to cause great concern to the pork industry throughout the world.[1] There has been a marked increase of clinical respiratory signs in 18- to 20-week-old growing-finishing pigs, a syndrome now described as the porcine respiratory disease complex (PRDC).[1] A number of infectious agents are widely considered to be the primary etiological agents of swine respiratory disease. However, noninfectious airborne contaminants in farms, i.e. dust, endotoxin (ET, part of the cell wall of gram-negative bacteria) and peptidoglycan (PG, part of the cell wall of all bacteria, particularly gram positive), may also be important contributing causes to these syndromes. Airborne contaminants have been shown to activate airway epithelial cells, alveolar macrophages (AM), and neutrophils, which can lead to a loss of the protective effect of AM and other lung defense mechanisms.[2–8] Dust (particularly the respirable fraction [< 5 µm]), bacterial particles, and ET concentrations appear to be lower in segregated early weaned (SEW) nurseries and all-in–all-out (AIAO) grower barns compared to conventional nurseries,[9] suggesting that pigs in SEW nurseries and AIAO facilities are exposed to lower concentrations of airborne contaminants.

A preliminary study was conducted to determine the short-term effect of the farm environment, which included exposure to airborne contaminants and disease agents, on pig health (Jolie R, et al. *Proc AASP Ann Meet.* 1998;377–378). One group of pigs was raised according to conventional procedures on a farm with a dusty environment and high prevalence of respiratory disease. A second group of pigs from the same farm was weaned at 2 weeks of age and was raised in an isolation facility. Five-week-old farm pigs had a higher proportion of neutrophils in blood and bronchoalveolar lavage fluid (BALF) (indicative of an ongoing inflammatory process) and decreased superoxide anion generation compared to pigs in the isolation facility. However, these differences disappeared by 10 weeks of age. Despite the high prevalence of respiratory disease in the herd, no specific macro- or microscopic respiratory lesions were found in any of the pigs at necropsy (10 weeks of age). The lack of differences between the two groups of 10-week-old pigs might indicate that the conventional farm environment does not affect pigs of this age. In addition, necropsy of 10-weeks-old pigs might have been too early to evaluate respiratory disease, and respiratory problems might have been more severe in the presence of virulent infectious agents, such as porcine reproductive and respiratory syndrome virus (PRRSV). The present follow-up study was conducted to further address these questions in pigs from a farm with a history of respiratory disease and PRRSV.

Material and methods

Farm and pigs

This follow-up study was conducted during the winter of 1997–1998 in a 75-sow, farrow-to-finish confinement herd. The farm consisted of one farrowing room, two nursery rooms, two grower barns, and one finisher barn. The farrowing, nursery, and grower units were mechanically ventilated, and the finisher was naturally ventilated with automated curtains. Hygiene and sanitation were above average.

The farm had a history of respiratory problems and had experienced an outbreak of PRRS almost 2 years before the study began. Three months before the start of this study, 77% of tested grower pigs were found to be serologically positive for PRRSV (by ELISA), indicating that PRRSV was still active in the grower barn. At slaughter checks, finishing pigs demonstrated a 95% prevalence of gross pneumonic lesions, 15% prevalence of pleuritis, and 15% prevalence of atrophic rhinitis.

Sixteen pigs (eight gilts, eight barrows) from four litters were randomly selected in the farrowing room and were eartagged at 1 week of age. At 14 days of age, pigs were assigned either to:

- an ISO group (n = 8), which was weaned on the day of selection and moved to the Animal Research Center (School of Veterinary Medicine, University of Wisconsin). The ISO pigs were medicated upon arrival by intramuscular (IM) injection of 4 mg per kg ceftiofur (Naxcel®, Pharmacia & UpJohn Inc.; Michigan) for 3 consecutive days. They were fed a specialized 21%–23% crude protein (CP) diet for early-weaned pigs (Land O' Lakes Inc.; Minneapolis, Minnesota) until 4 weeks of age. From 4–10 weeks of age, the pigs were fed a standard 18% CP nonmedicated corn-soybean meal diet, followed by a 16% CP nonmedicated corn-soybean meal diet throughout the finishing period.
- a Farm group (n = 8), which remained at the farm. The Farm pigs received ceftiofur starting at 14 days of age for 3 consecutive days, were weaned at 21 days of age, and were moved to one of the nursery rooms. They were raised according to standard farm procedures, with an 18% CP fortified grower and 16% CP finisher feed. The nursery diet contained 90 g per ton carbadox (Mecadox®, Pfizer Animal Health; Lee Summit, Missouri), and tylosin (Tylan®, Elanco; Indianapolis, Indiana) was mixed at 100 g per ton in the grower diet for the first 3–4 weeks, which was followed by 30 g per ton bacitracin methylene disalicylate (BMD®, ALPHARMA Inc.; Chicago Heights, Illinois) until slaughter.

Each group was balanced for gender, weight, and litter. Neither Farm nor ISO pigs received any vaccinations or antibiotic injections.

Environmental sampling

The Farm environment was sampled at the time of each BALF and blood sampling and only in the area in which the study pigs were housed at the time of sampling. The ISO environment was sampled 1 day before or after BALF and blood sampling.

Inhalable (up to 100 µm in particle size) and respirable (< 5 µm) dust samples were collected with an IOM inhalable sampler (SKC, Eighty Four; Pennsylvania) and a respirable cyclone, respectively.[10] All filters were stored in a polystyrene tube (Falcon® 2051, Beckton-Dickinson; Lincoln Park, New York) at 4°C and were shipped to Wako Pure Chemicals Ltd. (Japan). The filters were extracted and the extracts of the filters were analyzed for ET and PG, courtesy of M. Tsuchiya and A. Takaoka. Results were expressed as ng per m^3.[11]

Ammonia and carbon dioxide was measured using Auer detector tubes attached to a hand operated vacuum pump (Kwik-Draw Detector Tube pump, Mines Safety Appliances; Pittsburgh, Pennsylvania). Results were expressed as ppm.

Bronchoalveolar lavage technique

Bronchoalveolar lavages were performed on anesthetized pigs at 5, 7.5, 10, 15, and 20 weeks of age, using techniques previously described.[10] Briefly, pigs were anesthetized by IM injection with 4.4 mg per kg xylazine, followed by 4.4 mg per kg tiletamine and zolazepam (Telazol®, Fort Dodge Laboratories, Inc.; Fort Dodge, Iowa).[10] A custom-made, reusable, autoclavable, flexible endotracheal catheter (91 cm long, 3 mm internal diameter) (Bivona Medical Technologies; Gary, Indiana) was inserted into a lung lobe via the larynx and was kept in place with an inflated cuff. A different catheter was used for each pig. The lungs were washed with a total of 60 mL sterile phosphate buffered saline. All the recovered fluid was pooled and the total volume was recorded. An average of 72% of the fluid was recovered.

Analysis of BALF fluid

Total cell count, cell differentiation, and isolation

A 1-mL aliquot of pooled and filtered BALF fluid was collected for total cell counts (Coulter Electronics; Hialeah, Florida) and a cytocentrifugal smear (8 minutes, 300 × g) was prepared for cytology (Cytospin 2, Shandon Inc.; Pittsburgh, Pennsylvania) as previously described.[10]

Cells in the BALF were concentrated by centrifugation of the remainder of the sample (15 minutes, 400 × g, 4°C). The cell pellet was washed twice in Hanks balanced salt solution without Ca^{2+} and Mg^{2+} ($HBSS^{2-}$, Gibco BRL; Grand Island, New York), followed by centrifugation (15 minutes, 400 × g, 4°C). If the BALF fluid was blood tinged, the second wash was replaced by a hypotonic lysis of red blood cells with KCl and cold $HBSS^{2-}$, followed by centrifugation. After the second wash or lysis, the remaining cell pellet was resuspended in 1 mL of HBSS with Ca^{2+} and Mg^{2+} ($HBSS^{2+}$, Gibco BRL). A cell count of the concentrated cells was performed with an automated cell counter. The cells in this sample were used in the phagocytosis and superoxide anion generation assay. Cell viability was determined by the trypan blue exclusion assay and averaged 95%.

Phagocytosis assay

Phagocytic activity of AM was evaluated by mixing 2×10^6 lavage cells in 1 mL RPMI (Sigma Chemical Company; St. Louis, Missouri) with 10:1 fluorescent beads per cell (Fluospheres® 1 µm, Molecular Probes; Eugene, Oregon). Cells were incubated for 90 minutes at 37°C while rotating. At the end of the incubation, cells were centrifuged

(5 minutes, 1000 × g), washed in RPMI, and resuspended in 1 mL RPMI with 100 µL of 4% paraformaldehyde to fix cells. Phagocytosis was verified with a fluorescence microscope. The green fluorescence of the AM population was measured with a flow cytometer (Epics-Profile II, Coulter Electronics)and transformed logarithmically. Forward lightscatter and 90° light scatter were used to gate on AM. Results were expressed as the total percent of AM phagocytosing one or more beads (total phagocytosis).

Superoxide anion generation assay

Superoxide (SO) anion generation was measured as cytochrome C reduction to assess the activity of AM oxidoreductase.[12] Phorbol myristate acetate (PMA, 0.1 mg per mL, Sigma)-induced SO synthesis was determined in 4.5×10^6 lavage cells per mL in 100 µL $HBSS^{2+}$ by measuring the velocity of reduction of ferric cytochrome C (Sigma) at 550 nm (30 minutes, 37°C). Changes in optical density were converted to molar concentrations using an extinction coefficient of 18.5×10^3 L per mol per cm. All samples were run in triplicate and compared to a simultaneously analyzed control, which contained superoxide dismutase (SOD, Sigma). Results were expressed as nmol per min per 10^6 cells. Since the lavage cells consisted of other cells besides AM, the results were corrected for 100% AM and 100% viability.

Blood sampling and analysis

Prior to each BAL procedure, a 1-mL aliquot of peripheral blood was collected from the vena cava in an EDTA Vacutainer® tube for total white blood cell and differential cell counts, as previously described.[10,13]

An additional 10-mL blood sample was collected for serology. Serology was performed at Bayer Diagnostic Laboratory (Worthington, Minnesota) with the exception of the *Mycoplasma hyopneumoniae* serology (performed by Dr. Eileen Thacker at Iowa State University, Ames, Iowa). ELISA assays were used for *Actinobacillus pleuropneumoniae* (positive >2) serotypes 1, 5, and 7, swine influenza (SIV) (positive >1), PRRSV (S:P < 0.4 negative, S:P > 0.4 positive), and *Haemophilus parasuis* (positive >1). A microagglutination assay was used for *Streptococcus suis* (positive >1/8). An optical density value > 0.2 was considered positive for *M. hyopneumoniae*.

Weight data

Pigs were weighed prior to each BALF and blood sampling and average daily gain (ADG) was calculated.

Evaluation of lungs and snouts

Farm and ISO pigs were slaughtered on the same day to ensure blinding of the investigator during the scoring of lungs and snouts. At necropsy, lungs and pleura were examined macroscopically and the volume of lesions was scored on a scale of 0%–100%.[14] A cross-section of the snouts was scored on a scale from 0–9 for atrophic rhinitis lesions.[14]

Statistical analysis

Statistical analysis was performed using Systat 7.0 (SPSS Inc.; Chicago, Illinois) and SAS (SAS Institute Inc.; Cary, North Carolina). Descriptive statistics were calculated for all variables. A general linear model (GLM) for repeated-measures procedure was used for each variable to estimate the effect of age and the interactive effect of age and group/location**.** Differences between Farm and ISO pigs within a time period were measured with a multiple comparison of means method in the GLM procedure.

Differences between Farm and ISO for airborne contaminants, pneumonia lesions, atrophic rhinitis score, and ADG were determined by a two-sample Student's t-test.

Results

Overall, Farm pigs were exposed to higher concentrations of the different airborne contaminants compared to the ISO pigs (Table1).

Ammonia ($P < .005$) and CO_2 ($P < .05$) concentrations were significantly higher in the Farm than in the ISO environment. Ammonia concentrations in the Farm averaged 5.3 ppm (SD ±2.9) and ranged from 2–9.5 ppm, with the highest concentration in grower and finisher barns. The CO_2 concentration in the Farm environment averaged 1900 ppm (SD ±710) and ranged from 1200–3200 ppm, with the highest concentration in the nursery. In contrast, ammonia concentration in the ISO room average 1.2 ppm (SD ±0.8). Carbon dioxide concentration in the ISO room averaged 425 ppm (SD ±253) and ranged from 300–750 ppm. The temperature in the Farm averaged 22°C in the nursery and 17°C in the grower and finisher barns. The temperature in the ISO unit averaged 25°C for the early-weaned pigs and was gradually reduced to 18°C when the pigs reached finisher age.

Serum titers at 5 weeks of age were positive for PRRSV in four of eight pigs in both Farm and ISO groups (maternal antibodies). A suspect (1:16) antibody titer was measured in three of eight Farm pigs and one out of eight ISO pigs. All Farm pigs had seroconverted to PRRSV by 15 weeks of age, but the ISO pigs remained negative (Table 2). At 20 weeks of age, seven of eight Farm pigs also had antibody titers against *M. hyopneumoniae*. Although clinical signs were not monitored, coughing was observed in Farm pigs from 10 weeks of age until slaughter.

Total white blood cell and neutrophil concentrations in peripheral blood were significantly higher in Farm pigs than in ISO pigs (Table 3). Neutrophil concentrations decreased significantly as the Farm pigs grew older, and were higher at all ages compared to ISO pigs (Tables 3 and 4).

Superoxide anion generation was significantly higher in Farm pigs than in ISO pigs. Superoxide anion increased in both groups of pigs as they aged (Table 4), but the increase was numerically greater in the Farm pigs. Between 10–20 weeks of age, the total phagocytosis activity was greater in the ISO pigs than in the Farm pigs (Tables 3 and 5).

Total white blood cell counts in BALF did not differ between ISO and Farm pigs, but were influenced by age of pigs (Tables 3 and 6). The

Table 1

Concentrations of airborne contaminants in Farm and ISO facilities. Dust samples in the Farm facility were only collected in the area that the pigs were housed at the time of bronchoalveolar lavage and blood sampling. The Farm samplings were 1 day apart from samplings in the ISO facility.

		Airborne contaminants				
	Number of samples	Dust (mg/m^3)	Peptidoglycan (PG) (µg/m^3)	PG/Dust (µg/mg)	Endotoxin(ET) (ng/m^3)	ET/Dust (ng/mg)
Inspirable						
Farrowing	1	5.0	ND	ND	99.3	19.9
Nursery	1	8.8	7.7	0.88	122.8	14.0
Grower	1	2.4	1.8	0.76	0.4	0.2
Finisher	1	9.4	3.7	0.40	6.0	0.6
Farm*	4	6.4	4.4	0.68	57.13	8.7
ISO†	7	2.6 (0.3-4.7)	0.71 (0.35-1.17)	0.45 (0.22-1.15)	0.15 (0.08-0.32)	0.09 (0.03-0.24)
P value‡		.042	.015	.402	.052	.059
Respirable						
Farrowing	1	0.457	0.24	0.53	0.7	1.51
Nursery	3	0.400	0.34	1.03	4.7	9.6
Grower	2	0.429	0.16	0.49	0.6	1.7
Finishing	3	0.272	0.24	0.94	0.7	3.0
Farm*	9	0.388	0.263	0.822	2.0	4.3
ISO	6	0.097 (0.0-0.22)	0.06 (0.04-0.10)	3.7 (0.19-17.9)	2.4 (0.1-13.4)	16.2 (0.1-85.7)
P value‡		.002	.002	.224	.893	.351

* Farm is average of samplings in farrowing, nursery, grower, and finisher.

† n=7 samples throughout the study period of 15 weeks, results are presented as the mean (range) throughout the study period.

‡ *P* value was determined with the two sample Student's T-test.

Table 2

Number of 15- and 20-week-old Farm and ISO pigs with positive serology results for different respiratory pathogens

	Location of pigs	
	Farm (n=8)	ISO (n=8)
	Number of pigs with positive antibody titer of a total of 8 pigs tested	
15 weeks		
Actinobacillus pleuropneumoniae 1,5, or 7	0	0
Haemophilus parasuis	4	3
Mycoplasma hyopneumoniae	0	0
Porcine respiratory and reproductive syndrome virus (PRRSV)	8	0
Swine influenza	0	0
Streptococcus suis	5	2
20 weeks		
Actinobacillus pleuropneumoniae 1,5, or 7	0	0
Haemophilus parasuis	7	7
Mycoplasma hyopneumoniae	3	0
Porcine respiratory and reproductive syndrome virus (PRRSV)	8	1
Swine influenza	0	0
Streptococcus suis	3	0

Serology was performed at Bayer Diagnostic Laboratory, Worthington, MN, USA, with the exception of *Mycoplasma hyopneumoniae*. The latter was tested courtesy of Dr. E. Thacker at the Veterinary Medical Research Institute, Ames, IA, USA.

Table 3

Statistical results (*P* values) for each hematology and BALF variable. Results were obtained with a general linear model for repeated measures, estimating the effect of age and the interactive effect of age and group/location (i.e., ISO or Farm).

		Within group/location	
	Between group/location	Age	Age × group/location
Hematology			
White blood cells	.046	.099	.348
Neutrophils	.001	.017	.315
Lymphocytes	.428	.059	.120
BAL			
Superoxide anion	.004	.000	.000
Total % phag. AM	.031	.000	.035
Total cells	.203	.000	.048
AM	.001	.000	.885
Lymphocytes	.009	.000	.022
Neutrophils	.001	.006	.002

highest total cell count measured in both Farm and ISO pigs was at 10 weeks of age. The AM was the major cell in the BALF of Farm and ISO pigs and percentage of AM was significantly higher at all ages in ISO pigs (Table 6). Age and group influenced the percentage of lymphocytes, and percentage of lymphocytes was significantly higher in 15- and 20-week-old Farm pigs (Tables 3 and 6). The percentage of neutrophils was also significantly influenced by age and group of pigs, and was higher in the Farm pigs at all times with the exception of the sample taken at 15 weeks of age (Tables 3 and 6).

No macroscopic lung lesions were found in the 20-week-old ISO pigs. Macroscopic pneumonic consolidations were observed in five of eight Farm pigs. The lung volume involved was 23%, 20%, 6%, 1.5%, and 1% for these five pigs. The lung lesions were located in the anterior lung lobes and were suggestive of a subacute *M. hyopneumoniae* infection. Some scar tissue, indicative of previous infections, was also observed in the lungs of all Farm pigs.

The mean atrophic rhinitis score was 0.9 in the ISO pigs and 1.9 in the Farm pigs. One Farm pig had a score of 6 on a scale of 0–9.

The starting weight averaged 4.9 kg in both groups. The ADG from 5 weeks of age to slaughter in ISO pigs was 952 g and in Farm pigs was 794 g ($P < .0001$).

Table 4

Total cell and cell differential ($\times 10^3/\mu L$) in peripheral blood of Farm and ISO pigs (n=8).

	Farm		ISO	
	Mean	95% CI	Mean	95% CI
5 weeks of age				
White blood cells	16.5	2.2	14.4	3.6
Neutrophils	7.6	1.7	5.3	0.9
Lymphocytes	7.6	1.0	8.2	2.9
7.5 weeks of age				
White blood cells	17.2	3.4	18.0	3.5
Neutrophils	9.5	2.1	7.7	3.1
Lymphocytes	6.9	1.9	9.3	1.3
10 weeks of age				
White blood cells	18.3	3.0	15.7	1.6
Neutrophils	9.8	1.6	5.3	1.1
Lymphocytes	7.1	1.7	9.5	1.5
15 weeks of age				
White blood cells	20.8	6.3	15.8	2.6
Neutrophils	9.0	1.9	6.1	2.8
Lymphocytes	10.5	5.2	7.8	1.6
20 weeks of age				
White blood cells	20.5	2.5	18.0	2.8
Neutrophils	7.3	1.9	5.4	1.4
Lymphocytes	10.7	2.0	11.0	1.9

Discussion

The main objective of this study was to examine the long-term effect of farm environments—including exposure to airborne contaminants, disease agents, and other stress factors—on animal health by raising

Table 5

Superoxide anion generation and phagocytosis activity in bronchoalveolar lavage fluid of Farm and ISO pigs (n=8).

	Farm		ISO	
	Mean	95% CI	Mean	95% CI
5 weeks of age				
Superoxide (nmol/min/10^6 cells)	0.735	0.168	0.831	0.063
Total phagocytosis*	43.4%	25.4%	46.4%	13.4%
7.5 weeks of age				
Superoxide	1.037	0.199	0.665	0.157
Total phagocytosis	46.5%	12.0%	44.5%	15.9%
10 weeks of age				
Superoxide	0.946	0.260	1.100	0.122
Total phagocytosis	44.3%	9.8%	64.6%	7.4%
15 weeks of age				
Superoxide	1.543	0.293	1.183	0.105
Total phagocytosis	38.5%	5.8%	47.4%	6.3%
20 weeks of age				
Superoxide	2.094	0.260	1.669	0.165
Total phagocytosis	60.0%	6.4%	70.5%	8.2%

* Total phagocytosis – the total percentage of alveolar macrophages phagocytozing one or more fluorescent latex beads

Table 6

Total white blood cells, alveolar macrophage, lymphocyte and neutrophil proportions in bronchoalveolar lavage fluid of Farm and ISO pigs (n=8).

	Farm		ISO	
	Mean	95% CI	Mean	95% CI
5 weeks of age				
Total white blood cells ($\times 10^5$/mL)	4.5	1.2	9.0	1.6
Alveolar macrophages	87.6%	4.1%	96.1%	1.8%
Lymphocytes	4.6%	2.5%	2.3%	1.1%
Neutrophils	7.3%	3.5%	1.5%	0.9%
7.5 weeks of age				
Total white blood cells ($\times 10^5$/mL)	9.9	3.1	11.5	1.9
Alveolar macrophages	77.5%	10.1%	85.9%	5.7%
Lymphocytes	6.5%	3.5%	5.1%	1.7%
Neutrophils	15.9%	8.4%	8.9%	5.1%
10 weeks of age				
Total white blood cells ($\times 10^5$/mL)	14.2	4.3	12.7	1.7
Alveolar macrophages	73.6%	7.2%	83.5%	5.8%
Lymphocytes	9.2%	3.2%	8.8%	2.8%
Neutrophils	17.1%	6.9%	7.5%	4.1%
15 weeks of age				
Total white blood cells ($\times 10^5$/mL)	9.1	2.7	9.1	2.1
Alveolar macrophages	78.6%	6.6%	89.3%	2.7%
Lymphocytes	17.2%	6.9%	7.4%	2.5%
Neutrophils	4.0%	2.7%	3.1%	2.2%
20 weeks of age				
Total white blood cells ($\times 10^5$/mL)	7.9	2.7	7.5	0.9
Alveolar macrophages	78.3%	12.3%	92.3%	2.4%
Lymphocytes	11.1%	6.1%	4.5%	1.7%
Neutrophils	10.5%	11.1%	2.8%	1.3%

pigs from the same farm in the conventional farm environment or in an isolated facility.

Environment

The airborne contaminant concentrations tended to be higher in the Farm environment than in the ISO environment, which was consistent with our findings in the preliminary study. Within the farm, the highest inhalable dust concentrations were measured in the nursery and finishing rooms, while the respirable dust fractions were higher in the farrowing, nursery, and grower barns. The highest endotoxin and PG contaminations were measured in the farrowing and nursery rooms. The distribution of the inhalable and respirable dust and ET concentrations was consistent with previous reports.[15–17] There have been no comparable reports on concentrations of PG. The dust and PG concentrations in the isolation room increased with increasing age of the pigs, suggesting that the older pigs contributed more airborne contaminants to the environment. A similar observation was not made for ET concentrations, probably because the room was cleaned daily and fecal material was removed. This was in agreement with other studies conducted in this isolation room.[10] The results of the airborne contaminants in the Farm and ISO environment were based on a small number of samples collected throughout the course of the study. Dust, endotoxin, and PG concentrations varied on a daily basis and the available data only reflected the contamination at the time of sampling.

Pig health

As in our preliminary study (Jolie R, et al. *Proc AASP Ann Meet.* 1998;377–378), the overall results suggested that the ISO environment was associated with improved health of the pigs. Farm-raised pigs had lower AM proportions and higher neutrophil and lymphocyte proportions in BALF than ISO pigs, which has also been reported by others.[18–20] The neutrophil proportion in BALF was higher in the Farm pigs than in the ISO pigs at all ages. Increased neutrophils in BALF are considered an indicator of respiratory tract infection.[21,22] The higher peripheral neutrophil concentrations and total white blood cells in the Farm pigs probably reflected the cell composition in the BALF.

The Farm pigs became infected with PRRSV between 10–15 weeks of age while they were housed in the grower barn. After initial infection, PRRSV replicates in the alveolar macrophages (AM) and induces cell lysis, resulting in a decreased defense of the respiratory tract against other respiratory pathogens (Thacker E, et al. *Proc AASP Ann Meet*. 1998;1:351–356). Continuous replication of the virus in the AM produces interstitial pneumonia and provides an entry for the virus into the circulation.[23] In the Farm pigs, the decrease of the actual number of AM between 10 and 15 weeks of age was likely due to the PRRSV infection. In addition, PRRSV might have been potentiated by a concurrent *M. hyopneumoniae* infection (Thacker E, et al. *Proc AASP Ann Meet*. 1998;1:351–356). Macroscopic lung lesions suggestive of *M. hyopneumoniae* and *M. hyopneumoniae* antibody titers were observed in 20-week-old Farm pigs. *Mycoplasma hyopneumoniae*-induced pneumonia is recognized by infiltration of the lung tissue with mononuclear cells primarily consisting of lymphocytes and macrophages (Thacker E, et al. *Proc AASP Ann Meet*. 1998;1:351–356). In our study, Farm pigs had increased lymphocytes in BALF at 15 weeks age and an influx of neutrophils at 20 weeks of age. Asai, et al.,[24] observed a mixture of AM, neutrophils, and lymphocytes 27 and 32 days after an experimental infection with *M. hyopneumoniae*. *Mycoplasma hyopneumoniae* and PRRSV are considered primary pathogens in the PRDC.

Phagocytosis and superoxide anion generation were measured as indicators of AM function and results in the Farm pigs may also have been influenced by the PRRSV and *M. hyopneumoniae* infection. Phagocytosis and superoxide anion generation are important in the killing of microorganisms.[25,26] The increasing superoxide anion concentration in the PRRSV-positive Farm pigs is in contrast to the work of Solano, et al., who observed reduced AM bacterial uptake and superoxide production in the later stages of a PRRSV infection.[27]

The phagocytic activity was increased in 20-week-old Farm pigs, but was still lower than in the ISO pigs. This finding is consistent with observations made by Caruso, et al.,[28] who reported a reduced phagocytic activity in AM from pigs infected with *M. hyopneumoniae* and a secondary pathogen. However, it is not known whether a concomitant infection of PRRSV and *M. hyopneumoniae* has the same effect. Overall, the effect of a concomitant PRRSV and *M. hyopneumoniae* infection on the AM function has not been investigated.

In absence of disease agents, the AM function might also be influenced by other environmental stressors, such as airborne contaminants. The ISO pigs, which were raised in a disease-free environment, had no pneumonic lesions but appeared to have activated AM. Similar observations have been made by our group in previous studies under the same conditions.[10] Chapes, et al.,[29] reported that conventionally housed healthy pigs have detectable activated AM, as measured by cytotoxicity of tumor cells, due to environmental conditions. Spontaneous cytotoxicity of AM was not found in AM from pigs raised under germ-free conditions.[30] As can be concluded from the airborne contaminant data, the ISO pigs in our study were also exposed to environmental challenges, which might have influenced the AM activity. An infection with PRRSV and *M. hyopneumoniae* probably further altered the AM function, as in the Farm pigs.

The results suggest that blood and BALF parameters can be influenced by factors in the environment, other than disease agents. The Farm pigs developed pneumonic lesions before 20 weeks of age and were serologically positive for PRRSV and *M. hyopneumoniae*. The ISO pigs were not exposed to PRRSV or *M. hyopneumoniae*, did not have any antibodies against PRRSV and *M. hyopneumoniae*, and lacked respiratory lesions. However, AM in BALF appeared to be activated, probably by exposure to the airborne contaminants in the ISO room. The possibility that a combined infection of the ISO pigs with PRRSV and *M. hyopneumoniae* could have further altered the cellular composition of the BALF and AM function requires further research.

Implications

- Housing pigs in a clean, disease-free, isolated, and low-stress environment positively influences respiratory health of pigs.
- It is difficult to raise pigs in an airborne-contaminant-free environment.
- Minimal airborne contamination, as found in ISO housing, is still sufficient to induce cellular changes in BALF. An infectious agent, such as PRRSV and/or *M. hyopneumoniae*, could further alter the cellular composition of the BALF and AM function.

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