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Original Article

Assessment of Swine (*Sus scrofa domesticus*) Alveolar Macrophage Viability Associated with Heavy Metal Air Pollutants

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Abstract

The impact of air pollution on airway cellular defense of pigs raised under backyard, semi-commercial and commercial conditions was investigated. Flame atomic absorption spectrometry was used to analyze Cadmium (Cd) and Lead (Pb) while manual cold vapor atomic absorption spectrometry was applied to evaluate Mercury (Hg) contents of dust particles from farms. Tests for viable counts of swine alveolar macrophages (SAM) that simulate animal responses to air pollutants, microbiological techniques for the recovery of bacterial cells and evaluation for cellular function were undertaken. Results showed that air pollution was accompanied by significant high levels of Pb and Cd. Exposure of SAM to air pollutants induced significant reduction in cell viability and the reduction was substantially contributed by the duration of exposure and the farm source of SAM and air pollutants. Significant differences in colony counts of *P. multocida* were recorded as effects attributed by SAM exposure, the duration of exposure to air pollutants and the farm origin of the tested SAM and air pollutants. Data that revealed high recovery counts of *P. multocida* were taken as a measure for altered microbicidal action of SAM against the bacterium. *In vitro* interaction of backyard-obtained SAM and air pollutants for 6 h does not modify the killing or microbicidal action of SAM on *P. multocida* but prolonging the SAM and air pollutant interface beyond 6 h may eventually weaken the microbicidal action of the cells which allows incessant yielding of high bacterial recovery counts and failure of SAM to counteract this process.

Keywords: air pollution, cadmium, lead, macrophage, mercury, swine

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Introduction

Air pollution presents a serious threat to animal and human health by facilitating the entry of pathogens that challenge the respiratory system. The polluted air does not only facilitates entry of pathogens that challenge the respiratory system but it also carries heavy metals with known toxic effects to animals and humans. The buildup of heavy metals that remain persistent in living tissues is an impending cause of alarm because these are neither degraded by chemicals nor by any biological process (Ferner, 2001; and Thakur, 2006). A deteriorating air quality exists in the Philippines as indicated by the attainment of an Environmental Performance Index (EPI) of 77.9% which has been linked to many morbid cases of bronchitis in Metro Manila (EMB, 2008).

Contamination of the environment with heavy metals through air, water and the food chain has aroused concerns both on the local and global scale. A multitude of information describe the ecological (Shilutong et al., 2000; Fakayode and Olu-Owolani, 2003; Perrins et al., 2003; Kotwal et al., 2005) and toxicological (Braunwald et al., 2001; Jarup, 2003; Duruibe et al., 2007; Neustadt and Pieczenic, 2007; Akinola et al., 2008; Islam et al., 2008; Cruz et al., 2009; Islam et al., 2010; Obeid et al., 2010; and Kaplan et al., 2011) upheaval associated with heavy metals. Ecological and toxicological reports point up the detection of heavy metals like lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), zinc (Z), manganese (Mn) and iron (Fe) in air, water, animal and plant samples.

Accounts intended to describe host cellular defenses against air pollutants of the respiratory system remain ambiguous. The involvement of alveolar macrophages in the clearance of respiratory pathogens and particulate matters in the lungs, the triggering of the ciliary escalator system (Patton et al., 1997) and the complex interplay of innate and adaptive cellular responses that ward off bacteria and inflammation in the respiratory tract have all been presented contrary to reports on damage and deterioration of alveolar macrophages (Patton et al., 1997) and abrogation of physiological processes for pathogen elimination (Braunwald et al., 2001).

The impact of a deteriorating air quality on animal health has to be taken with great concern in time that the economy of the country relies on animal production. Limited information defining the effect of a weakening air quality on animal health exists. Cells of the respiratory tract that are involved in the clearance of bacterial infection described by researchers (Riley et al., 2005; and Lanome et al., 2009) may be studied to define the effect of toxic components of the air on animal health.

This study was initiated to investigate the heavy metal components of dust particles collected from piggery farms that operate under commercial, semi-commercial and backyard conditions. Relative to this, experiments that examined the efficiency of host respiratory defense system measured in terms of the ability of viable alveolar macrophages to exert cytotoxic effects even after exposure to air pollutants on a potential respiratory pathogen were comparably monitored. Identifying the impact of air pollution on innate respiratory cellular defense is an important reference in advocating preventive interventions against air pollution-instigated respiratory infections.

Materials and Methods

Collection of air pollutants in animal production sites: Six pig farms (2 Backyard type, Farm A; 2 Semi-commercial type, Farm B; and 2 Commercial type, Farm C) which are on full production operation for more than 3 years, with feed mills, located in areas with draft wind, dusty and without tunnel ventilations were chosen as sites for collection of air pollutants. Five pieces of sterile gauze panels mounted in pre-fabricated galvanized iron rods were installed in the 6 farms within a day at the start of the experiment. The rods were fixed 4 to 5 ft high on wooden poles and placed in 5 areas of each farm where draft wind and dust could be collected for 1 wk. Panels previously installed were collected after 1 week, placed in plastic disposable sterile wraps and soaked in 150 ml DW for 2 h before collection of washings. The samples were pooled in sterile beakers, labeled to represent the farm of origin and kept frozen at -80°C until ready for use in the succeeding experiments.

Flame Atomic Absorption Spectrometry for Detection of Cadmium (Cd) and Lead (Pb) in air pollutants: Cd and Pb were evaluated using the methods of Csuros and Csuros (2000). Briefly, 100 ml of each sample was placed to dry in a crucible dish in an oven. The sample was placed in a furnace and heated at 100°C up to 450°C. The sample was allowed to cool at room temperature for 8 h before pre-ashing in an infrared radiation lamp. Pre-ashing was done by continuously heating the sample in a furnace at 200°C, then to 250°C up to 450°C. The sample was again allowed to cool for 8 h as before, moistened with distilled water and allowed to evaporate in a hot plate. The ash sample was again heated in a furnace at 200°C up to 450°C for 1 to 2 h until it became completely white to gray in color. Five (5) ml 6.0 M HCl was added to the sample, allowed to evaporate in a hot plate before dissolving the residue in 10 to 30 ml 0.1 M HNO. The sample was allowed to stand for 1 to 2 h before determining the amount of Cd and Pb. Levels of Cd and Pb were determined by comparing the sample with a standard reagent when read in the Flame Atomic Absorption Spectrometer. The detection limit of the assay for Cd and Pb was 1 to 5 parts per billion (ppb) from any tested sample.

Manual Cold-Vapor Atomic Absorption Spectrometry for Detection of Mercury (Hg): Methods of Cherian and Gupta (1990) in detecting Hg were adapted. One hundred (100) ml of each sample was placed in a digestion flask before adding 20 pcs of boiling stones. Cold DW (5 ml) was added to the sample in a flask during digestion. Sufficient heat was applied for 6 min to effect a low initial boil while a strong boil for 10 min followed to finalize the digestion. The flask was removed from heat before adding two drops of 30% H_2O_2 and washing with 15 ml DW. The digested material was transferred to a 100-ml volumetric flask and compared with a standard reagent when read in

the Cold Vapor Atomic Absorption spectrometer. The foregoing method could reportedly detect 1 part per billion (ppb) of Hg from any tested sample.

Collection of alveolar macrophages: Lungs (4 lung samples from each type of piggery system) from healthy finisher pigs (5 months old) that had been raised from the farms, ready for slaughter and had passed ante-mortem examination were chosen as sources of lung samples. Lung samples were perfused with 150 to 200 ml RPMI 1640 (Sigma, Germany). Tracheal washings were allowed to drain and were collected in sterile flasks before pooling samples to represent each type of farm. Alveolar macrophages from the tracheal washings were washed twice by centrifugation at 3000 rpm for 20 min. Pooled cells collected from the pigs of commercial farms contained 96% macrophages with 95% viability score after trypan blue exclusion test. Tracheal washings of the pigs from semi-commercial farms contained 97% macrophage population with 95% viability. Cells of tracheal washings from the pigs with backyard origin had 97% macrophage population and a viability rate of 96%. The cells were collected and re-suspended in RPMI 1640 at a density of 1.5 x 10^6/ml before plating.

Evaluation of effect of air pollutants on alveolar macrophage viability: The alveolar macrophages were plated in 4-well tissue culture plates (Nunclon, USA). Provision for each type of farm included 9 plates that covered treatments of alveolar macrophages reacting with 300 µl air pollutant suspension (T_1), untreated alveolar macrophages (T_2) and RPMI alone (T_3) all set in 3 replicates, in treatment duration of 6, 12 and 24 h of incubation. The samples were incubated at 37°C in a humidified incubator (Model-9300, Wakenyaku Co. Ltd. Japan). After the incubation of cells at specified duration of treatment with the suspensions of air pollutant, cell supernatants were collected in sterile tubes, centrifuged at 3000 rpm for 20 min, stained with Trypan blue, counted and computed based on mean cell counts x dilution factor x 10^4/ml. The computed values were considered as residual viability of cells in response to treatment with air pollutants from the farms. To rule out factors other than air pollutants contributing to cell death, computation for cell death percentages were undertaken both in treated and untreated cells.

Microbicidal effect of air pollutant-treated alveolar macrophages on the test bacterium Pasteurella multocida: A pure culture of *Pasteurella multocida* (*P. multocida*) was obtained from the College of Veterinary Science and Medicine, Central Luzon State University. *P. multocida* had a cell density of 1.5 x 10^9 CFU/ml and was used as a test cell in evaluating microbicidal effect of swine alveolar macrophage (referred to as cytotoxic effect of the cells after exposure to air pollutants) against *P. multocida*.

Air pollutant-treated alveolar macrophages from the replicated plates in the previous experiment were collected at the completion of 6-, 12- and 24-hr treatment and pooled to represent each farm. Air pollutant-treated cells were washed separately by centrifugation at 3000 rpm for 20 min, re-suspended in RPMI 1640 at a density of 1.4 x 10^6 cells/ml.

In synchronization with this experiment, request and arrangement were previously made with a slaughterhouse located in a different town for the collection of alveolar macrophages from healthy finisher pigs that came from a farm source different from the study sites and had passed ante-mortem evaluation before slaughter. The collected lung samples were perfused with RPMI 1640 as in the previous step, the collected cells were plated at a density of 1.5 x 10^6 cells/ml and utilized as air pollutant-untreated alveolar macrophage.

P. multocida (200 µl/well) was allowed to react with the air pollutant-treated and air pollutant-untreated alveolar macrophages during incubation at 37°C for 6, 12 and 24 h in a humidified incubator. The alveolar macrophages were collected from wells at the end of each treatment period for evaluation of alveolar macrophage viability in the presence of *P. multocida*. Cells were stained with Trypan blue, only the cells with clear cytoplasms on the hemocytometer were counted. Viable counts were taken as mean cell count x dilution factor x 10^4/ml.

P. multocida was collected from wells after *in vitro* interaction of SAM and air pollutants as well as the untreated SAM at indicated time intervals and inoculated into Brain heart infusion (BHI) agar. McFarland turbidity and standard plate counting were applied to determine colony counts of the organism.

Statistical Analysis: Data were analyzed using 3 x 3 Factorial in Randomized Complete Block Design (RCBD). Least Significant Differences (LSD) was used to test differences among treatments. P-values lower than 0.01 and 0.05 were considered significant.

Results

Heavy metals in air pollutants in pig farms: Evaluation of dust particles from the 3 types of piggery farms was marked by the presence of heavy metals. The levels of heavy metals in the samples are summarized in Table 1. Air pollutants from the backyard pig farms had significantly higher Cd contents ($P<0.05$) while those of the semi-commercial and commercial farms had comparable lower values. Pb levels in the 3 types of farms were comparable and values ranged from 5.0 to 13.2 ppm. Air pollutants from the commercial farms had significantly higher Hg levels ($p<0.01$) compared to the samples from backyard and semi-commercial farms, which were comparable.

Effect of air pollutants on alveolar macrophages: The impact of air pollution on animal health could be better understood by observing closely the interaction of swine alveolar macrophages (SAM) and suspension of air pollutants containing the heavy metals in vitro. Data in Table 2 demonstrate that the viable numbers of macrophages exposed to suspensions of air pollutants (T1) for 6, 12 and 24 h were significantly lower ($p<0.01$) than the viable counts of macrophages not exposed to air pollutant suspensions (T2). Data also show that the number of viable SAM exposed to suspension of air

pollutants were significantly different (p<0.05) at specified periods of exposure, which was not noted in the untreated cells. SAM collected from the pigs of backyard farms exposed to suspensions of air pollutants had significantly higher viable counts (p<0.05) observed at 24 h but not in the other time points. These data explicate that the air pollutants, which have the capacity to carry the above-mentioned toxic metals, reduced the viable counts of SAM upon contact and the reduction was influenced by the duration of exposure and that SAM that came from the commercial farms registered the least number of viable cells.

Reduction in the viable counts of SAM can be considered as a damaging effect of air pollutants on animals and this can be verified by monitoring the mortality % of these cells. Data in Table 3 provide evidence that SAM exposed to suspensions of air pollutants had significantly higher mortality % (p<0.01) compared to the untreated cell counterparts. The differences were significantly noted at indicated periods of observation (p<0.05). The mortality of SAM collected from commercial farms, either exposed or not to suspensions of air pollutants that were also derived from the same farm source had significantly higher mortality % (p<0.01) compared to the data from the other farms.

Microbicidal action of swine alveolar macrophages post-exposure to air pollutants: Data that showed the reduction in the viable numbers of SAM in corroboration with higher mortality rates of cells exposed to the suspensions of air pollutants were further tested in terms of their capability to counteract

Table 1 Mean concentration of toxic metals in 3 pig production systems

Heavy Metals (ppm)	Farm A (Backyard)	Farm B (Semi-commercial)	Farm C (Commercial)	Permissible limits*
Cd	0.820 (± 0.016)^{a}	0.0700 (± 0.125)^{b}	0.600 (± 0.082)^{b}	0.500
Pb	13.200 (± 0.163)	7.700 (± 0.082)	5.000 (± 0.810)	5.000
Hg	0.008 (± 0.001)^{b}	0.009 (± 0.001)^{b}	0.010 (± 0.001)^{a}	0.100

Values represent mean (± SEM) levels of heavy metals (ppm) in air pollutants collected from the 3 farms. *, Levels permitted by international standards (AJFAI, 2009). $^{a, b}$, Significant differences in Cd (p <0.05) and Hg (p<0.01) across farms.

Table 2 Comparative residual viable number of swine alveolar macrophage (cells/well) related to exposure and non-exposure to air pollutants from piggery farms

Treatments	Farm A (Backyard)	Farm B (Semi-commercial)	Farm C (Commercial)
6 h			
T1	1,449,079 (7.41)$^{b, A}$	1,448,420 (110)$^{b, A}$	1,446,145 (449)$^{b, A}$
T2	1,449,205 (75)^{a}	1,449,265 (15)^{a}	1,449,145 (15)^{a}
T3	0^{c}	0^{c}	0^{c}
12 h			
T1	1,448,387 (52)$^{b, B}$	1,446,715 (165)$^{b, B}$	1,443,715 (165)$^{b, B}$
T2	1,449,010 (60)^{a}	1,448,605 (345)^{a}	1,448,140 (90)^{a}
T3	0^{c}	0^{c}	0^{c}
24 h			
T1	1,447,720 (14)$^{b, C, X}$	1,444,578 (8)$^{b, C, Y}$	1,441,607 (337)$^{b, C, Z}$
T2	1,448,425 (75)^{a}	1,448,350 (30)^{a}	1,447,435 (225)^{a}
T3	0^{c}	0^{c}	0^{c}

Values represent mean (± SEM) viable numbers of swine alveolar macrophages (1.5 x 10^{6} cells/well) post-exposure to air pollutants from the 3 farms at indicated duration of interaction. T1, swine alveolar macrophages (SAM) exposed to air pollutants. T2, SAM not exposed to air pollutants. T3, RPMI medium alone. $^{a, b, c}$, differences between viable counts of SAM as affected by exposure and non-exposure to air pollutants (p<0.01). $^{A, B, C}$, differences between viable counts of SAM as affected by duration of exposure (p<0.05). $^{X, Y, Z}$, differences between viable counts of SAM as affected by farm of origin (p<0.05).

the proliferation of the test pathogen *P. multocida* in the presence of air pollutants. Data in Table 4 exhibit significant differences in the colony counts of *P. multocida* as affected by the exposure and non-exposure of SAM to air pollution (p<0.01), where higher counts of the test bacterium were recovered from wells that contained SAM exposed to suspensions of air pollutants. Significant differences in the colony counts of *P. multocida* were also noted (p<0.01) as affected by the duration of exposure of SAM to air pollutant suspensions, where the 24-hr interaction period tailored higher bacterial colony counts. The colony counts of *P. multocida* were also significantly different as an effect of the farm origin of the tested SAM and air pollutants (p<0.01). It is emphasized, however, that SAM obtained from the backyard farms, which were allowed to react for 6 h with air pollutant suspensions coming from the same source, may exert a greater killing or microbicidal action on *P. multocida* that gave rise to lower recovery counts of the bacterium, an observation that was comparably mounted by SAM not exposed to air pollutants, but contrary to the higher bacterial recovery counts derived from SAM exposed to air pollutants for 12 or 24 h.

Discussion

The results demonstrated that air pollutants in swine production farms are characterized by high levels of Pb and Cd, where Cd in the three farms and Pb levels in the backyard and semi- commercial farms exceeded those of international standards (US-EPA, 2012). The presence of heavy metals in dust particles is alarming as this can facilitate heavy metal deposition in many biological substrates that can cause adverse effects in humans and animals. Smoke, use of Cd-containing products (Jarup, 2003), worm treatments for swine (Hardy et al., 2008) have been described as sources of Cd. High amounts of Pb are reportedly contracted from plants processed as feed for animals (Abdullah Alkhalaf et al., 2010). This raises the contention that faulty manure management and improper disposal of rarely-recycled Cd-containing materials seen in farms can both contribute to Cd and Pb accumulation.

Table 3 Comparative mortality % of swine alveolar macrophages related to exposure and non-exposure to air pollutants from piggery farms

Treatments	Farm A (Backyard)	Farm B (Semi-commercial)	Farm C (Commercial)
6 h			
T1	3.40 (0.0005)$^{a, C, Y}$	0.0001)$^{a, C, Y}$	3.69 (0.0006)$^{a, A, X}$
T2	3.39 (0.0005)$^{b, A, Y}$	3.36 (0.0002)$^{b, B, Y}$	3.43 (0.0007)$^{b, A, X}$
T3	0^{c}	0^{c}	0^{c}
12 h			
T1	3.44 (0.0004)$^{a, B, Z}$	3.55 (0.0001)$^{a, B, Y}$	3.65 (0.0001)$^{a, A, X}$
T2	3.39 (0.0004)$^{b, A, Y}$	3.43 (0.0002)$^{b, A, X}$	3.46 (0.0005)$^{b, A, X}$
T3	0^{c}	0^{c}	0^{c}
24 h			
T1	3.49 (0.0009)$^{a, A, Y}$	3.69 (0.0001)$^{a, A, X}$	3.63 (0.0006)$^{a, A, X}$
T2	3.44 (0.0005)$^{b, A, X}$	3.44 (0.0001)$^{b, A, X}$	1.92 (0.0015)$^{b, B, Y}$
T3	0^{c}	0^{c}	0^{c}

Values represent mean (±SEM) mortality % of swine alveolar macrophages (SAM, 1.5 x 10^6 cells/well) post-exposure to air pollutants from the 3 piggery farms. T1, SAM exposed to air pollutants. T2, SAM not exposed to air pollutants.T3, RPMI medium alone. $^{a, b, c}$, differences between mortality % of SAM as affected by exposure and non-exposure to air pollutants (p <0.01). $^{A, B, C}$, differences between mortality % of SAM as affected by the duration of exposure (p<0.05). $^{X, Y, Z}$, differences between mortality % of SAM as affected by the farm of origin (p <0.05).

Air pollutants from the three farms prepared as suspension of dust particles act by causing reduction in viable swine alveolar macrophages tested in vitro. The reduction in macrophages may depend on the duration of exposure to air pollutants and is favorably acquired by air pollution from a commercial farm source. Decrement on the viable counts of alveolar macrophages after in vitro exposure to suspensions of dust particles done in this study parallels the findings of others who established counts of macrophages post-exposure to copper and zinc-based particles and made account on the destruction and cytotoxicity of alveolar epithelia and macrophage cell lines (Lanome et al., 2009). Alveolar macrophage function tested both in vitro and in vivo with the use of soluble dust particles reportedly caused decrease in the percentage of cells

that phagocytosed particles (Soukup et al., 2000; and Renwick et al., 2001), which is in accord with the findings of Imrich et al. (2000) on the reduction in alveolar macrophage populations relative to the alteration of macrophage viability and abrogated activation of an inflammatory mediator identified as macrophage inflammatory protein (MIP-2). Inhibition of cell growth and cell death has been described in the work of Riley et al. (2005) as a response of lung-derived cell lines to different concentrations of heavy metals in combustion particulate materials of air pollutants.

The lower colony counts of recovered *P. multocida* from the air pollutant-exposed SAM with backyard farm origin demonstrate a bacterial clearing accomplished by a larger number of viable macrophages exposed to air pollution in a short period of time (6 h). The data at hand explain altered ability of macrophages to exert an ultimate killing effect against a pathogen following a previous exposure to dust particles. It is safe to deduce that the macrophages which were previously exposed to dust particle suspension in vitro may deteriorate easily, become incapable of exerting the microbicidal or killing action against a respiratory pathogen like *P. multocida* that comes in contact with it. Cell death demonstrated by the reduced viable counts of SAM against a gradual increase in *P. multocida* colony counts can be considered as a de-stabilized bactericidal action of alveolar macrophages against *P. multocida* in animals previously exposed to air pollution. This is probably an initial data for *P. multocida* to be isolated and grown after interaction with swine alveolar macrophages as very little is known about the culturability and viability of respiratory pathogens following phagocytosis and interaction with macrophages. Bitekov et al. (2000) measured the viability of *Mycobacterium tuberculosis* by MPN after serial dilution of the pathogen in a liquid medium, established the colony forming units (CFUs) after obtaining the bacterium from macrophages several days post-infection, with the application of appropriate recovery and resuscitation protocols.

The above findings provide a preliminary insight into the impact of heavy metals that accumulate in animals and cause abrogation of cellular defenses in the airways in response to air pollution. The data generated in the study can be used as reference in fortifying preventive measures against untoward conditions brought in by air pollution. Research work that recognizes pathological states related to a deteriorating air quality has to be undertaken as important components of animal health programs. It is suggested that metal analysis of tracheal fluids or lung tissues is also needed to confirm certain exposure to heavy metals in the lungs.

Table 4 Comparative colony counts of *P. multocida* (CFU/well) recovered after in vitro interaction of swine alveolar macrophage and air pollutants in comparison with unexposed SAM

Treatments	Farm A (Backyard)	Farm B (Semi-commercial)	Farm C (Commercial)
6 h			
T1	10,327,500 (1,750)$^{C, Z}$	20,056,000 (6000)$^{a, C, Y}$	50,005,000 (2500)$^{a, C, X}$
T2	10,353,000 (3000)	10,527,000 (1000)^{b}	12,039,750 (1251)^{b}
12 h			
T1	15,682,000 (3000)$^{a, B, Z}$	35,500,650 (840)$^{a, B, Y}$	65,002,000 (165)$^{a, B, X}$
T2	10,681,750 (3500)^{b}	11,251,000 (255)^{b}	10,003,200 (2800)^{b}
24 h			
T1	55,000,750 (250)$^{a, A, Z}$	195,001,550 (1449)$^{a, A, Y}$	290,002,500 (1000)$^{a, A, X}$
T2	18,004,750 (255)^{b}	20,002,500 (500)^{b}	20,002,250 (741)^{b}

Values represent mean (± SEM) colony counts of *P. multocida* post-interaction and non-interaction of swine alveolar macrophages (SAM) with air pollutant suspensions from the 3 farms at indicated duration of interaction. T1, colony count of *P. multocida* recovered from SAM and air pollutant suspensions interaction. T2, colony count of *P. multocida* recovered from untreated SAM. $^{a, b}$, differences between colony counts of *P. multocida* recovered after exposure and non-exposure of SAM to air pollutants (p <0.01). $^{A, B, C}$, differences between colony counts of *P. multocida* recovered at specified duration of interaction of SAM with air pollutants (p <0.01). $^{X, Y, Z}$, differences between colony counts of *P. multocida* recovered after interaction of SAM with air pollutants from the same farm of origin (p <0.01). Alveolar macrophages (1.4 x 10^6 cells/well). *P. multocida* (300,000,000 CFU/well).

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บทคัดย่อ

การประเมินการมีชีวิตของแม็คโครฟาจในถุงลมจากสุกร (*Sus scrofa domesticus*) ที่มีความเกี่ยวข้องกับโลหะหนักที่เป็นมลพิษทางอากาศ

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ผลกระทบของมลพิษทางอากาศต่อการป้องกันระดับเซลล์ในทางเดินหายใจของสุกรที่เลี้ยงแบบหลังบ้าน กึ่งการค้า และสภาพแบบการค้าได้ถูกทำการศึกษา Flame atomic absorption spectrometry ถูกใช้เพื่อวิเคราะห์แคดเมียม (Cd) และตะกั่ว (Pb) ในขณะที่ manual cold vapor atomic absorption spectrometry ถูกใช้เพื่อประเมินปรอท (Hg) ที่มีอยู่ในอนุภาคฝุ่นจากฟาร์ม มีการทดสอบเพื่อนับจำนวนแม็คโครฟาจในถุงลมที่มีชีวิตของสุกร (SAM) ที่กระตุ้นการตอบสนองต่อมลพิษทางอากาศ การใช้เทคนิคทางจุลชีววิทยาสำหรับการหาเซลล์แบคทีเรีย และการประเมินหน้าที่ของเซลล์ ผลการศึกษาแสดงว่ามลพิษในอากาศมีระดับของตะกั่วและแคดเมียมที่สูงมาก การสัมผัสของ SAM กับมลพิษทางอากาศเหนี่ยวนำให้มีการลดลงอย่างมากของการรอดชีวิตของเซลล์ และการลดลงอย่างชัดเจนนี้มีสาเหตุจากระยะเวลาที่มีการสัมผัส และแหล่งของ SAM และมลพิษในอากาศ ความแตกต่างอย่างมีนัยสำคัญในการนับโคโลนีของ *P. multocida* ได้ถูกบันทึกไว้ เพราะผลกระทบนี้มีสาเหตุจากการสัมผัสของ SAM ระยะเวลาที่สัมผัสกับมลพิษ และที่มาของฟาร์มที่มีการทดสอบ SAM และมลพิษ ข้อมูลที่ได้แสดงถึงจำนวนของเชื้อ *P. multocida* ที่แยกได้นั้นถูกจัดเป็นการวัดสำหรับหน้าที่ในการฆ่าจุลชีพที่เปลี่ยนแปลงไปของ SAM ในการต้านแบคทีเรีย การมีปฏิสัมพันธ์ในหลอดทดลองของ SAM ที่ได้จากสุกรเลี้ยงแบบหลังบ้านกับสารมลพิษเป็นเวลา 6 ชั่วโมง ไม่ได้ปรับเปลี่ยนผลของการฆ่าหรือการฆ่าจุลชีพของ SAM ต่อ *P. multocida* แต่การยืดระยะเวลาของ SAM กับสารมลพิษนานกว่า 6 ชั่วโมงอาจทำให้หน้าที่ในการฆ่าจุลชีพของเซลล์อ่อนแอลงในที่สุด ซึ่งทำให้เพิ่มจำนวนของแบคทีเรียอย่างไม่หยุดหย่อน และความล้มเหลวของ SAM ในการต้านกระบวนการนี้

คำสำคัญ: มลภาวะทางอากาศ แคดเมียม ตะกั่ว แม็คโครฟาจ ปรอท สุกร

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