Relationship between Cattle Cellular Immunity and Endotoxin Levels in Dust from Cattle Housing Environment

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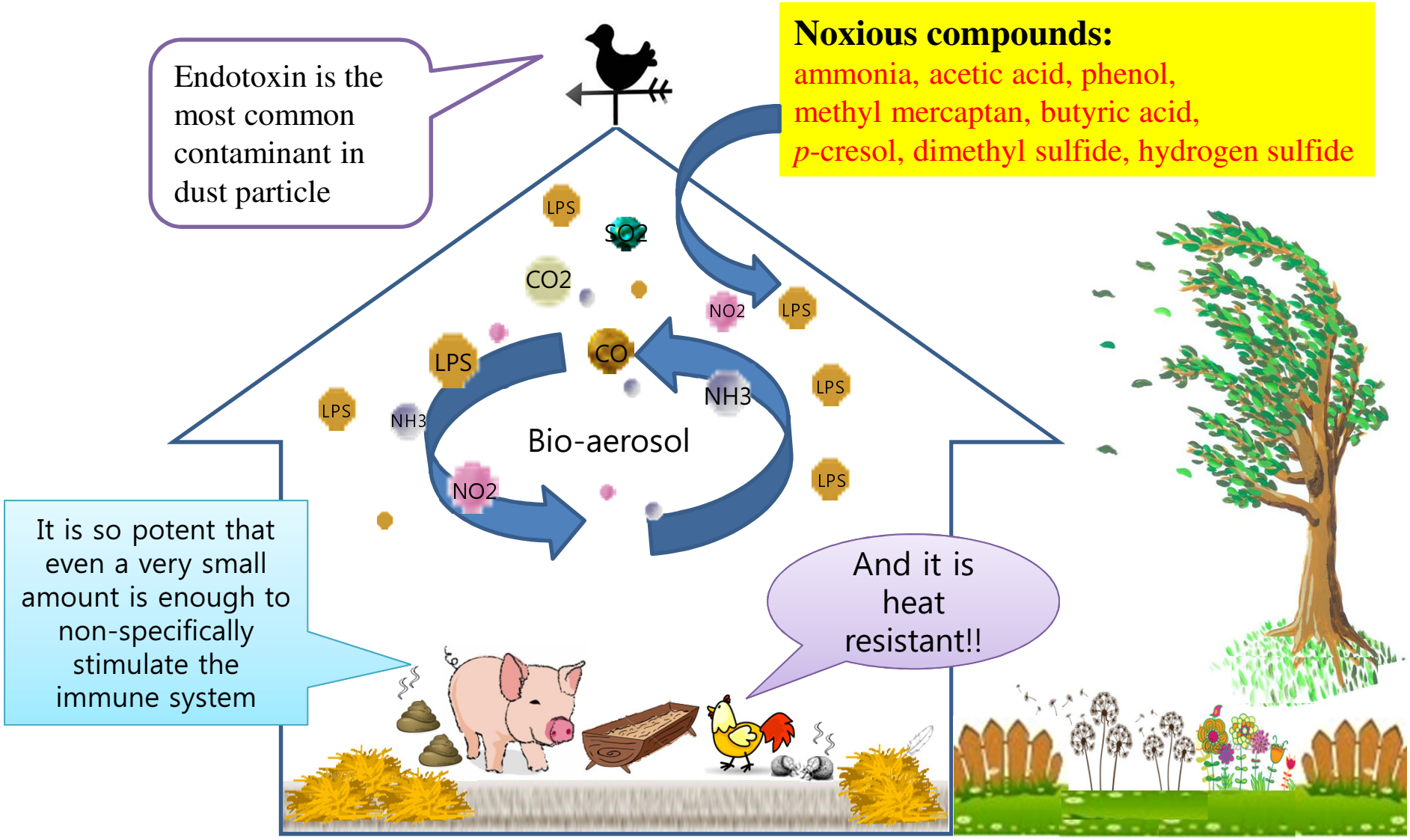
Kim, Hong-Do (1745-1806)

Biological and chemical hazards in indoor husbandry environment



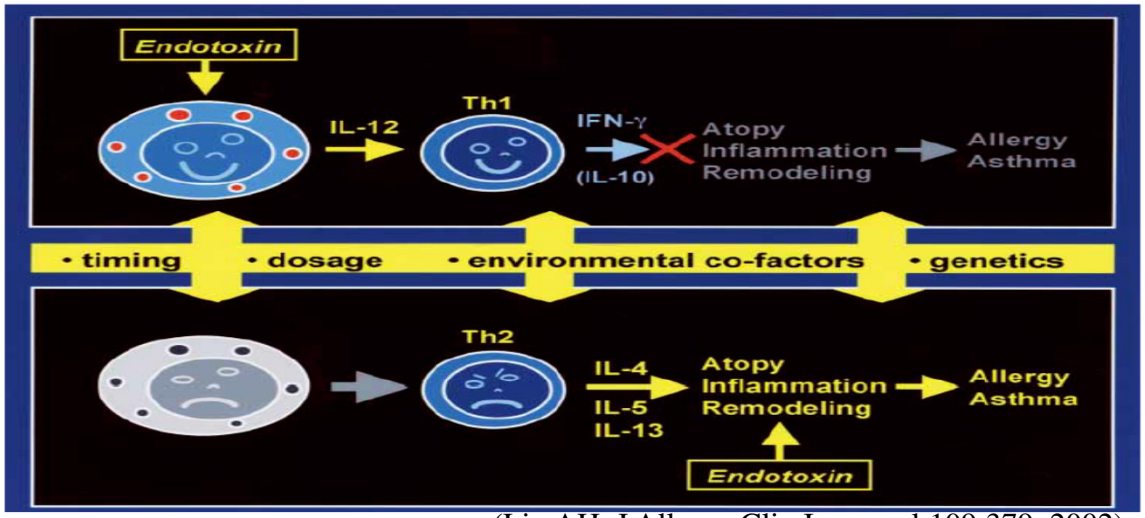
Biological hazards to animal husbandry workers

- Target population : dairy farmers, poultry farm workers, pig barn workers, animal handlers

- Chemical hazards :
  - **Acute and chronic dermal, ocular and respiratory diseases from exposure to ammonia, H_2S, CO, CO_2, SO_2, Nox**
  - **Dermatitis and respiratory tract irritation by detergents, disinfecting agents (formaldehyde)**

- Biological hazards :
  - **Primarily organic dusts from feathers, dander, feed and bedding, hay and grain, microorganisms (endotoxin)**
  - **Immunologically mediated diseases :organic dust toxicity syndrome, occupational asthma, chronic obstructive pulmonary disease, allergic alveolitis, hypersensitivity pneumonitis, farmer's lung disease, dermatitis**

- **Human**



(Liu AH, J Allergy Clin Immunol 109:379, 2002)

Endotoxin exposure has been recognized as a causative agent or contributing factor for various pulmonary illnesses including asthma, organic dust toxic syndrome, or chronic obstructive pulmonary disease

- **In animal (*in vivo* and *in vitro* experiments)**
  - Non-specific pathophysiological reactions, such as fever, changes in white blood cell counts, disseminated intravascular coagulation, hypotension, shock and death.
  - Up regulation of proinflammatory cytokine gene expression such as IL-1βs, IL-6, and IL-8 was also observed from chicken heterophils in the presence of LPS.
  - LPS induced anorexia, increased temperature, circulating TNFα and cortisol in swine.

Guideline for endotoxin exposure threshold level

No internationally agreed threshold level

Guidelines for 'no-effect' levels for environmental endotoxin.

Disease	EU/m^3
Toxic pneumonitis	2000
Airways inflammation	100
Systemic effects	1000

1) Sykes et al., 2011. Waste Management 31:423
2) Rylander. 1997. Int. J. Occup. Environ. Health 3 (suppl.):s32-s36

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Relationship between chicken cellular immunity and endotoxin levels in dust from chicken housing environments

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	BS	Broiler	BE	LK	Laying hen	LG
Total dust (mg/m^3)	0.53 ± 0.40		0.57[†]	1.03 ± 0.23		0.18 ± 0.06
Respirable dust (mg/m^3)	0.08 ± 0.05		0.06 ± 0.04	0.23 ± 0.10		0.09 ± 0.12
Endotoxin in total dust (EU/m^3)	103.6 ± 103.1		620.4	56.3 ± 45.2		682.2 ± 219.2
Endotoxin in respirable dust (EU/m^3)	1.75 ± 1.75		6.83 ± 1.22	0.21 ± 0.12		171.8 ± 200.8

[*]Data are expressed as the mean ± SD. [†]Dust sampling was performed at two different locations for each farm. However, total dust data for the BE farm was obtained from one sampler due to mechanical problems with the other sampler.

Table 4. Humoral or cellular immunity parameters for broiler chickens or laying hens*

	BS	BE	LK	LG
Plasma IgY (mg/mL)	1.47 ± 0.22	1.52 ± 0.50	5.63 ± 1.60	5.98 ± 0.93
INF-γ (pg/mL)†	17.15 ± 14.38‡	0.0 ± 0.0§	251.35 ± 143.57	47.06 ± 32.59
Plasma cortisol (ng/mL)	1.77 ± 0.51	2.17 ± 0.65	2.47 ± 0.79	5.51 ± 2.69

*Data are expressed as the mean ± SEM. †Peripheral mononuclear cells (10^6) were stimulated with 5 μg concanavalin A at 41°C for 72 h in a 5% CO_2 incubator and the culture supernatants were collected to measure interferon-γ (INF-γ) production. ‡Significant difference (*p* value): BS *vs.* BE (0.024). §INF-γ concentrations in all the culture supernatants were under the detection level.

Association of endotoxin level in dust from indoor housing environment with pigs' immune response

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Level of endotoxin in total or respirable dust collected from swine farms.

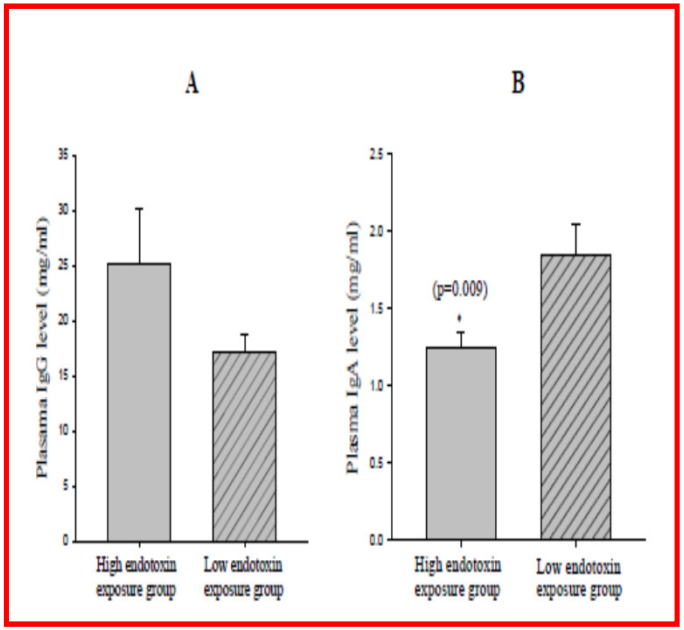
	High endotoxin exposure group	Low endotoxin exposure group	Statistical Significance (*p* value)
No. swine farms	5	5	
No. pig	604±429	362±153	n.s.
Stocking density (m^2/head)	1.15±0.25	1.23±0.33	n.s.
Age of pig (days)	138.8±13.3	118.4±23.4	n.s.
Total dust (mg/m^3)	0.87±0.60	0.58±0.35	0.0536
Respirable dust (mg/m^3)	0.67±0.21	0.32±0.36	0.0001
Endotoxin in total dust (EU/m^3)	443.18±480.56	13.05±12.07	0.0003
Endotoxin in respirable dust (EU/m^3)	7.22±4.86	1.03±1.22	0.0000

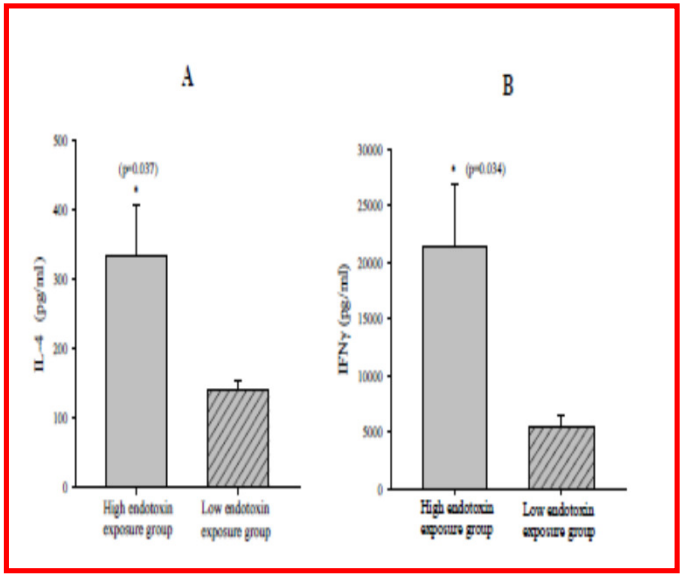
Endotoxin range in total dust: High endotoxin exposure group (47.1~1198.8 EU/m^3), Low endotoxin exposure group (0~29.6 EU/m^3). Data are expressed as the mean±SD.
n.s.: statistically not significant.

The Veterinary Journal submitted

Relationship (correlation coefficient) of demographic characteristics or concentration of endotoxin with various immunological parameters (*p* value in the parenthesis)

	Pig age (days)	Endotoxin in total dust (EU/m^3)	Endotoxin in respirable dust (EU/m^3)
IL-4 (pg/ml)	0.398 (0.006)		0.579 (0.000)
IFNγ (pg/ml)	0.622 (0.000)		0.697 (0.000)
TNFα (pg/ml)		0.706 (0.001)	-0.744 (0.000)
IL-12/23p40 (pg/ml)		0.663 (0.001)	-0.563 (0.009)
$CD1^+B$ cell (%)			0.555 (0.000)
WBC ($10^3/\mu l$)		0.496 (0.000)	
Platelet ($10^3/\mu l$)	-0.679 (0.000)		-0.361 (0.016)
Neutrophil ($10^3/\mu l$)		0.420 (0.003)	
Lymphocyte ($10^3/\mu l$)		0.351 (0.016)	
Monocyte ($10^3/\mu l$)		0.505 (0.000)	





The Research on Welfare of Farmers and Animals In Livestock Farms in Korea 2012-2016

(5 year project funded by Korean Rural Development Agency)

[그림 2] 계사내 미생물 포집 (left) 및 분진 포집 (right) 사진

Pigs → **'12~'13**

Brolier chicken
Laying hen → **'13~'14**

Beef /Dairy cattle → **'15~'16**

Safe and healthy rearing environments

→ Good welfare for both animals and workers

Immune parameters evaluated

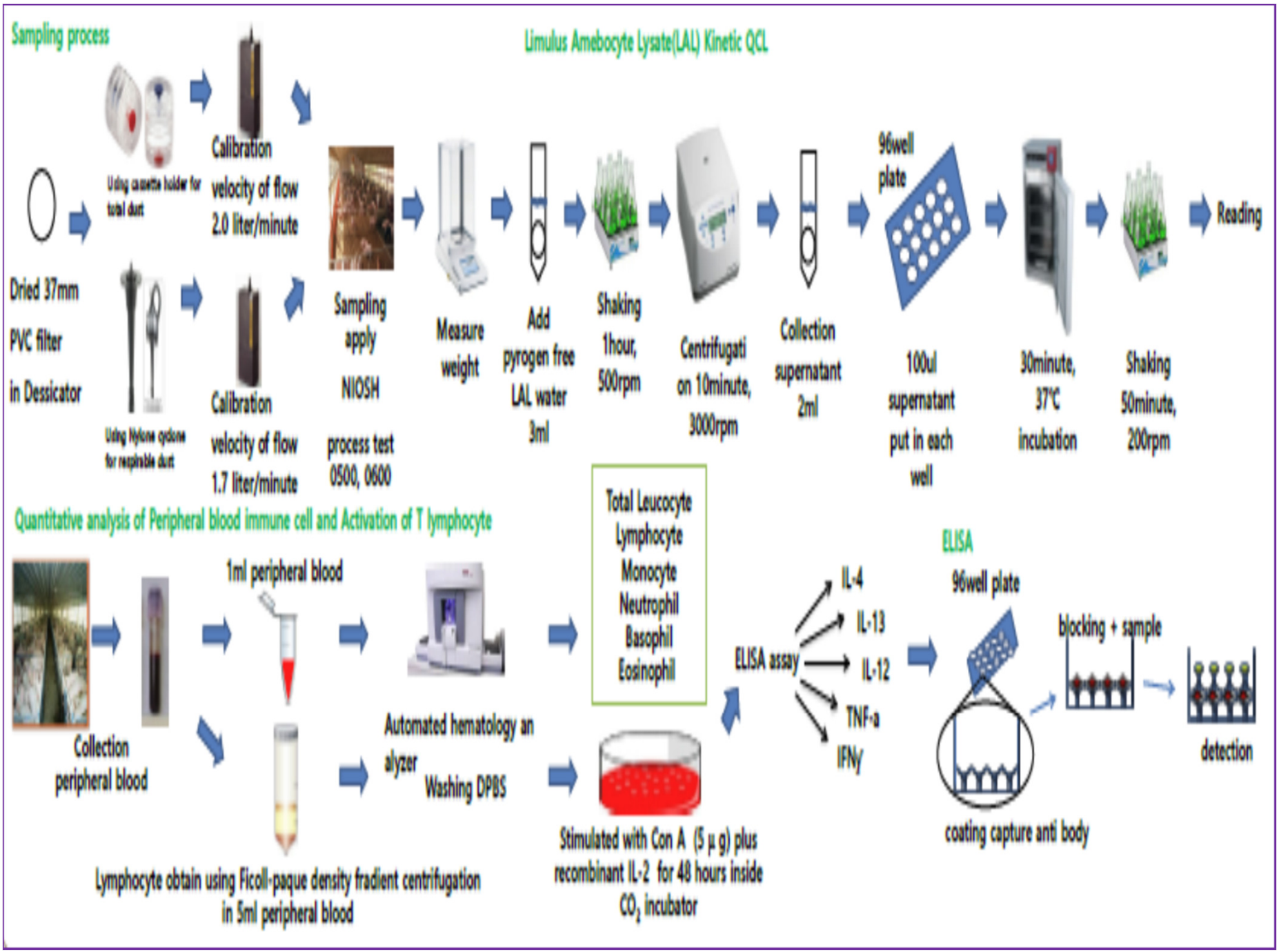
Category		Industrial animal
Immune function	Hematology	9 parameters for total WBC, RBC, lymphocyte, granulocyte, etc.
	Humoral immunity	Plasma total IgG
	Cellular immunity	- T cell activation with ConA for 72 hours: IL-4, IFNgamma
		- Phenotype analysis : CD21 B cell , CD4 helper T cell, CD8 cytotoxic T cell, WC1 γδ T cell, CD11b monocyte
Environmental monitoring	-dust collection indoor husbandry: Total dust, respirable dust -Level of endotoxin in dust -Level of aflatoxin in dust -isolation of pathogenic microorganisms (bacteria, fungi)	

Method for dust collection, endotoxin/aflatoxin measurement

- Concentration of total dust in indoor of the cattle farms was evaluated using PVC membrane filter (SKC, Eighty Four, PA, USA) with 2-stage cassette impactor at flow rate of 2.0 liters/min for 8 hours. Concentration of respirable dust (PM10) was determined using PVC membrane filter with 10 mm Dorr-Oliver nylon cyclone at flow rate of 1.7 liters/min for 8 hours. Dust samplings were done from two different locations (1/3 and 2/3 distance from the exit) at each farm.

- Endotoxin was extracted from the filters through adding 3 ml endotoxin-free Limulus Amebocyte Lysate (LAL) water (LAL Kinetic-QCL set, Lonza, Walkersville, MD, USA) with 5% Tween 20 followed by shaking for one hour at 350 rpm. Supernatants were collected and stored -80°C freezer until analysis. Endotoxin concentrations from the supernatants were evaluated following the company's instruction by a microplate spectrophotometer (Model Epoch™, Bio-Tek, Winooski, VT, USA).

- Aflatoxin concentration was determined using the Total Aflatoxin ELISA kit (EuroProxima, Netherlands).


Sampling process
Limulus Amebocyte Lysate(LAL) Kinetic QCL
Using cassette holder for total dust
Calibration velocity of flow 2.0 liter/minute
Dried 37mm PVC filter in Dessicator
Using Nylone cyclone for respirable dust
Calibration velocity of flow 1.7 liter/minute
Sampling apply NIOSH process test 0500, 0600
Measure weight
Add pyrogen free LAL water 3ml
Shaking 1hour, 500rpm
Centrifugation 10minute, 3000rpm
Collection supernatant 2ml
96well plate
100ul supernatant put in each well
30minute, 37℃ incubation
Shaking 50minute, 200rpm
Reading
Quantitative analysis of Peripheral blood immune cell and Activation of T lymphocyte
1ml peripheral blood
Collection peripheral blood
Automated hematology analyzer
Total Leucocyte
Lymphocyte
Monocyte
Neutrophil
Basophil
Eosinophil
Washing DPBS
Lymphocyte obtain using Ficoll-paque density fradient centrifugation in 5ml peripheral blood
Stimulated with Con A (5 μg) plus recombinant IL-2 for 48 hours inside CO_2 incubator
ELISA assay
IL-4
IL-13
IL-12
TNF-a
IFNγ
ELISA
96well plate
coating capture anti body
blocking + sample
detection

Korea's National Treasure No. 136
(Eternal smile from 1000 year past,
Shilla Dynasty) at GyeongJu, a UNESCO
Designated World Historic Site,
40 minutes distance from Daegu, Korea

Daegu GyeongJu

3rd largest beef cattle estate

Major characteristics of beef cattle* farms studied

Farms	No. cattle	Density (m^2/head) **	Average endotoxin (EU/m^3) in total dust	Average endotoxin (EU/m^3) in Respirable dust	Average aflatoxin (ng/m^3) in total dust	Average aflatoxin (ng/m^3) in respirable dust
BS	150	15.4	63	0.92	1.37	0.99
BM	350	28.9	178	2.46	1.21	1.21
BE	150	15.4	34	0.32	1.14	0.89
BJ	90	11.9	10	0.17	1.60	0.85
BG	160	14.4	0.6	0.40	1.51	1.10

*Age of cattle : no statistical difference among the farms (15~20 months)
**Korean standard for the pro-organic farming: 7.1 m^2/head
- No. cattle for blood collection per farm: 5
- Time for dust and blood collection: August, 2015

Received: 19 Feb. 2016, **Revised:** 13 Apr. 2016, **Accepted:** 8 Jun. 2016

Running title: Airborne microorganisms in livestock farms

Epizootiological characteristics of viable bacteria and fungi in indoor air from porcine, chicken, or bovine husbandry confinement buildings

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Microbial culture and species identification

- The culture plates were transported to Seoul National University, College of Veterinary Medicine for microbial cultivation and species identification.
- The TSA (trypticase soy agar) and BA (blood agar) plates were incubated at 37°C for 15-18 hours. The SDA (Sabouraud dextrose agar) plates were incubated at 30°C for 24 hours.
- After the incubation, colonies with different morphology, shape, smell or color were individually re-cultured in BA or SDA plates.
- After simple biochemical tests including Gram staining, oxidase test, and catalase test, microbial identification was carried out with VITEK®2 automated microbial identification system according to the instruction of the manufacturer (BioMerieux, Marcy l'Etoile, France).
- The colonies from SDA plates were also re-cultured and identified by fungal identification method based on colony morphology, characteristics of hyphae and spore after methylene blue or lactophenol blue staining.

Cattle sheds			
B1	*Acinetobacter iwoffi*	*B. cereus, B. licheniformis, E.faecalis, St. chrmogenes, St. lentus, St. vitulinus*	*Ca. albicans, Rhizopus spp.*
B2	*Acinetobacter iwoffi*	*B. cereus, B. licheniformis, K. rosea, St. chrmogenes, St. lentus*	*Ca. albicans*
B3	NI	*B. cereus, B. licheniformis, E.faecalis, St. chrmogenes, St. lentus*	*Ca. albicans*
B4	NI	*B. cereus, B. licheniformis, E.faecalis, St. chrmogenes, St. lentus, St. vitulinus*	*Ca. albicans*
B5	NI	*B. cereus, E.faecalis, St. chrmogenes, St. lentus*	*Ca. albicans*

Abbreviations: A.: *Alcaligenes, Ae.: Aerococcus, B.: Bacillus, C.: Cryptococcus, Ca.: Candida, E.: Enterococcus, G.: Gemella, Gr.: Granulicatella, K.: Kocuria, Kl.: Klebsiella, Pr.: Prototheca, S.: Sphingomonas, Sp.: Sphingobacterium, St.: Staphylococcus, T.: Trichosporon, V.: Vagococcus,*

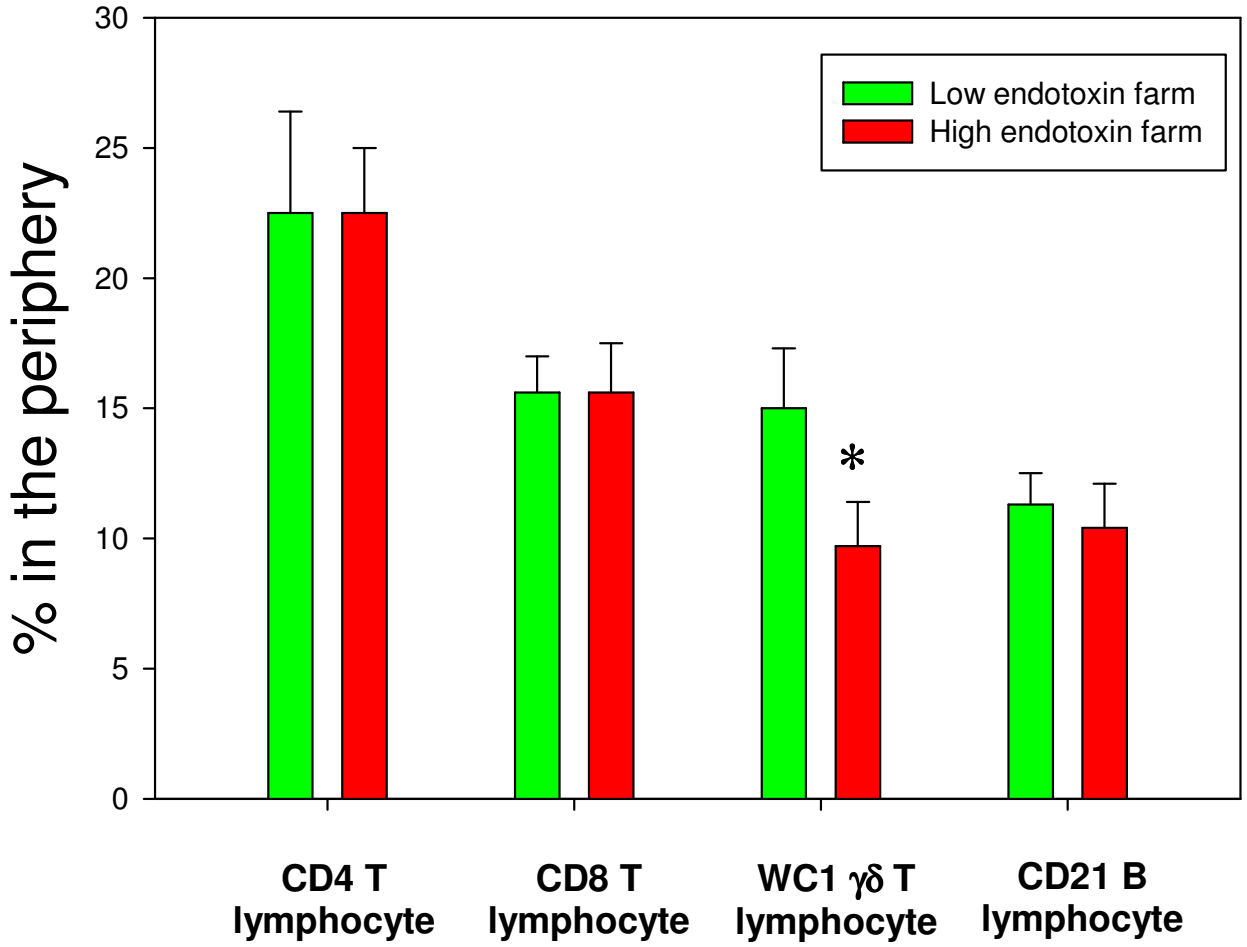
*NI: not isolated.

Comparison of hematologic parameters

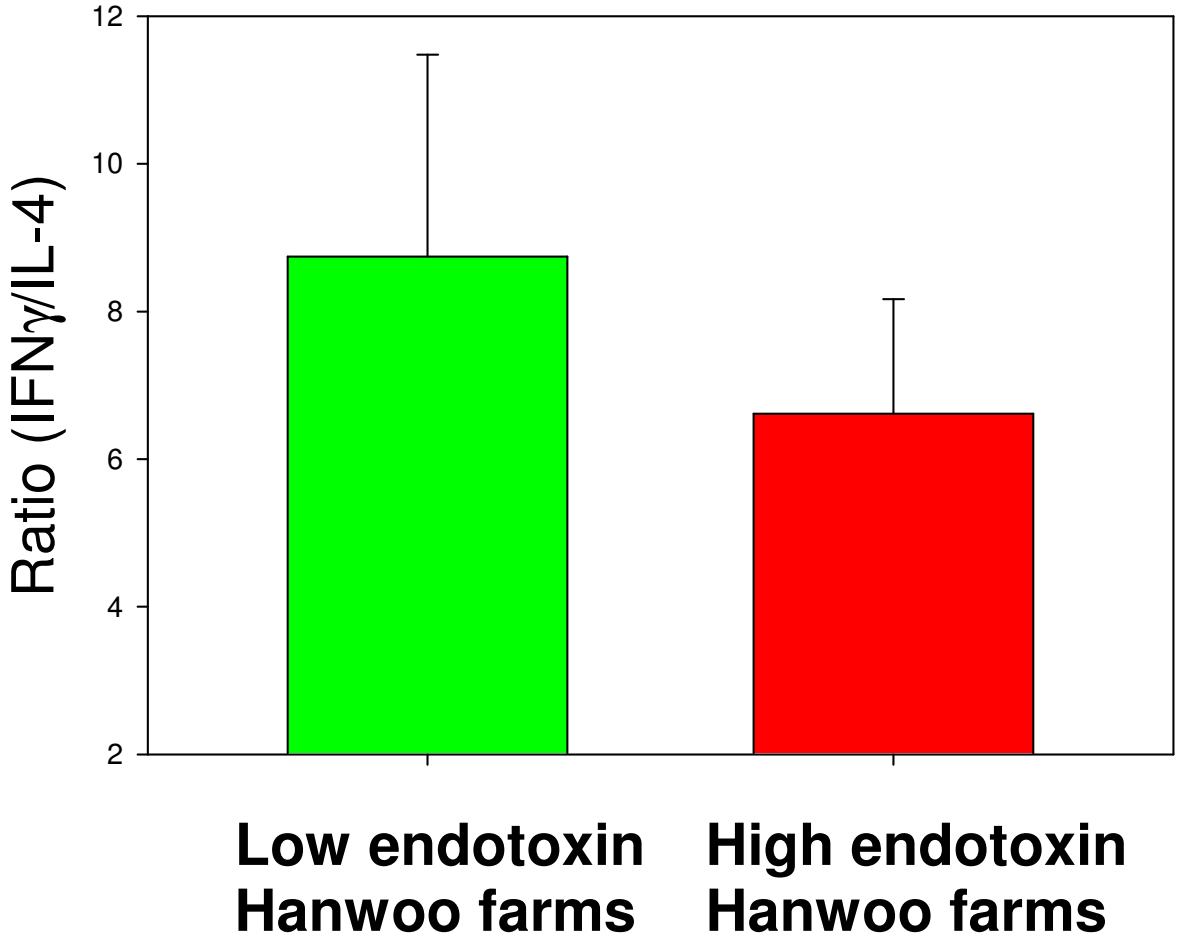
	High endotoxin exposure farms	Low endotoxin exposure farms	Significance (*p* value)
WBC (10^3/μl, 6.2~13.6)	7.52±0.32	11.37±0.47	0.0000
RBC (10^6/μl, 7.4~11.6)	8.10±0.38	9.03±0.24	0.039
Platelet (10^3/μl, 412~1003)	173.5±42.17	250.47±22.86	n.s.
Neutrophil (10^3/μl, 1.1~3.6)	3.25±0.34	3.50±0.28	n.s.
Lymphocyte (10^3/μl, 4.0~9.8)	2.69±0.32	5.89±0.25	0.0000
Monocyte (10^3/μl, 0.2~1.3)	0.39±0.05	0.39±0.08	n.s.
Eosinophil (10^3/μl, 0.0~0.7)	0.83±0.12	1.39±0.15	0.012
Basophil (10^3/μl, 0.0~0.3)	0.07±0.01	0.11±0.01	0.0003

Data are expressed as the mean±SE. n.s.: statistically not significant.

Distribution of major immune cells in the peripheral blood



Cytokine production from peripheral T cells



Summary and conclusion

- Overall, our results suggest a probable negative association between dust endotoxin levels and cell-mediated immunity in Korean beef cattle.
- Data on the cellular immunity could indicate lower immune defense against pathogenic challenge to cattle exposed higher concentration of endotoxin than cattle exposed to lower endotoxin level.
- Aflatoxin, which is detrimental to liver function, was also detected in the airborne dust of bovine farms.
- Systemic investigations should be further conducted to elucidate in details the association between husbandry environment and immunity in cattle, and also further evaluation for relationship with immunity of husbandry workers.

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH

Immunology for Public Health Lab.