candidates (*LTF*, *HGF*, *SERPINE1* and *CXCL11)* showing significant changes were proposed as potential biomarkers of JD. These biomarkers might be used for development of enhanced diagnostic tool of early stage of MAP infection in further study. This study was supported by the Rural Development Administration (PJ00897001) and the Research Institute for Veterinary Science, Seoul National University, Korea.

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P-044

Analysis of Inflammatory Cytokines Secreted by Raw 264.7 Cells with Stimulated *Brucella abortus* Mutants

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Introduction: Brucellosis is a zoonotic disease caused by the genus *Brucella* which can easily infect human and other animals. It makes serious damage of the host through its prolonged intracellular persistence in infected tissue while *Brucella* induces a low level inflammation as compared to other pathogens [1]. Cytokine has key roles inactivation of innate immunity and acquired immunity. Although roles of immune cells in the activation of acquired immunity are already known, it has been remaining to be solved to find essential roles of cellular components of *B. abortus* infection. On the basis of current understanding, production profiles of inflammation cytokines such as NO, IL-1β, IL-6, IL-12, IFN-γ, and TNF-α were compared with *B.* mutants. Moreover, it might be given a key to understand immune system and underlying mechanism.

Materials and Methods: RAW 264.7, mouse macrophages, cells were stimulated with *B. abortus* mutants generated by random mutation with transposon. Culture supernatants were collected from RAW 264.7 cells after stimulation with *B. abortus* mutants at different time intervals(0, 2hrs, 4hrs, 8hrs, 12hrs, 24hrs, 36hrs, and 48 hrs). Expression levels of inducible nitric oxide synthase (iNOS) were quantified by NO assay. Amounts of cytokine in the culture supernatant such as NO, IL-1β, IL-6, IL-12p70, IFN-γ,and TNF-α were quantified with ELISA.

Results: Nitric oxide was highly expressed after stimulating *B. abortus* wild type and *B.* mutants. Inflammatory cytokines were produced from Raw 264.7, mouse macrophage, cells after stimulation with the *B. abortus* mutants. Followed by production levels of inflammatory cytokines such as IL-1β, IL-6, IL-12p70, IFN-γ, and TNF-α were measured. As a result, expression levels of TNF-α and IL-6 were highly increased than other cytokines. Especially, IL-6 was highly expressed in RAW 264.7 with *Brucella* mutant infection compared to wild type infection after 24hrs. On the other hand, IL-1β, IL-12p70,and IFN-γ were showed both wild type and *B.* mutants under minimum expression levels of the quantified standard curve.

Conclusions: Though the TNF-α and IL-6 were showed high expression levels among 14 mutants out of 28 mutants, more analysis of other experiments is needed to find differences of the *B.* mutants and understand underlying mechanism of the pathogenesis as the further study.

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P-045

Analysis of Molecular Genomics of *Brucella abortus* Mutants Generated by Ez-Tn5™ pMOD™-3 Transposon

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Introduction: *Brucella abortus* is a well-known intracellular pathogen. Underlying mechanisms of the bacterial infection is very complicated to comprehend the pathogenesis though most of clues have been solved. Therefore, prevention and control of *B. abortus* infection is as ever problematic in animal and human. So, several methods including random mutation has been used to identify the mechanisms and find out the solution in prevention and control of the infection. Nevertheless, a continuous effort has been made, still the doors are locked in understanding of the mechanism.

Materials and Methods: Mutants of *B. abortus* were generated by random insertion of a transposon, Ez-Tn5™ pMOD™-3 <R6K*γori*/MCS> into chromosome. Characteristics of the mutants were investigated using biochemical test, growth curve, Biovar test, and finding virulence factors by PCR, PFGE, Southern blot and sequencing of the genes.

Results: *B. abortus* mutants were generated and insertion of the transposon was confirmed by Southern blot analysis with the transposon as a probe after PFGE. To understanding character of *B. abortus* mutants, at first, in the analysis of biochemical test, mutants of *B. abortus* were categorized 7 groups by ILATk, SUCT, and ELLM by comparison with wild type. Growth aspect was found variable depending on the mutants. Growth of the mutants were shown depending on the mutants in comparison of wild type. In biovar test, *B. abortus* wild type and all mutants were verified biovar type 1. PCR also was used for identifying virulence factors, and confirmed presence of virulence factors T4SS, PGK, and CGS without any change. The location of interrupted genes were identified in chromosome by sequence analysis of both sides of inserted transposon. Using sequencing information, twenty-eight genes were identified from the mutants by comparison with wild type of the bacteria, and made chromosomal mapping.

Conclusions: *B. abortus* mutants were characterized by

analyzing of their molecular genomics test. It might give a bottom line for further investigation. This work was supported by NRF (No. 2014R1A2A2A01007291).

P-046

Pesticide Poisoning of Domestic and Wild Animals in Korea

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Introduction: Pesticides are widely used to agriculture to prevent insects from making excessive inroads on the products of a farm and to inhibit outbreak of diseases carried by insects to human and animals. Animals were exposed to pesticides via ingestion of contaminated water, seeds and foliage. In this study, we investigated pesticides in the gastric contents of dead animals requested to the Animal and Plant Quarantine Agency (Gyeonggi-do, Korea) in Korea in 2014.

Materials and Methods: We analyzed residual pesticides in the gastric contents from the dead animals which were suspected pesticide poisoning based on the necropsy. Pesticides analysis was done using gas chromatography with flame photometric detector and mass spectrometry.

Results: A total of 234 gastric contents samples of 44 species were analyzed in this study and pesticides were determined in 25.6% of the total samples analyzed. Monocrotophos and phorate were the most common pesticides identified. Other organophosphates (i.e. diazinon, turbufos, parathion, phosphamidon, dichlorvos), organochlorines (i.e. endosulfan) and carbamates (i.e. carbofuran and methomyl) pesticides were also found in various concentrations from dead animals.

Conclusions: Poisonings by pesticides occur as a result of misuse or accidental exposure, but intentional killing of unwanted animals also occurs. In this study, results indicate a poisoning status of animals in Korea and suggest that pesticides poisonings will continue to be a cause of death in some animals in Korea. More attention should be paid to pesticide poisoning and future efforts to reduce the number of pesticide-related deaths are needed to help preserve the animals.

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P-047

Results of the Monitoring of Veterinary Drug and Chemical Residues in Domestic Meats in 2014

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Introduction: The Korean National Residue Program (NRP) for meats was established in 1996 to ensure the safety of livestock products and provided for guidance for effective implementation of testing. The NRP consists of three key factors for domestic meats: monitoring, surveillance and exploratory testing. Monitoring and surveillance testing programs are routinely implemented by 17 Animal Health Laboratory or the Health and Environment Laboratory of province (AHL) for domestic foods of animal origin. The NRP for meats apply to bovine, swine, chicken, duck, sheep, goat and horse, etc. at slaughterhouses and on farms. In this study, we investigated on the results of monitoring of domestic meat for the veterinary drug and chemical residues and change in trend of violation after the surveillance of the system in 2014.

Materials and Methods: Veterinarians in AHLs collect samples of urine or blood from livestock animals and screen for antimicrobial residues. Inspectors collect random samples of tissue (muscle, fat, kidney or liver) at the slaughterhouse and send the samples to AHLs to test for antibiotics, synthetic antimicrobials, hormones, and pesticides (Monitoring Plan). Inspectors take samples from individual suspect animals and suspect population animals at the slaughterhouse and send the samples to AHLs to test for penicillin, tetracycline, and quinolone antibiotics, synthetic antimicrobials, hormones and pesticides (Surveillance testing). On the basis of QIA method for residue analysis, veterinary drug residues were analyzed using a bioassay for screening purposes and by HPLC and LC-MS/MS for confirmation and quantification.

Results: The monitoring plan was conducted for 143 substances including 48 antibiotics, 59 synthetic antimicrobials, 2 hormones, 28 pesticides and 6 drugs such as Ractopamine, Clenbuterol, Azaperon, Carazolol, Phenylbutazone and Flunixin from 6 major species. The average violation rate in 2014 was 0.10% of 156,315 samples and 0.62% of 42,903 samples in monitoring plan and surveillance testing, respectively. The total violation rate was 0.21% from 199,218 samples tested in 2014. Violation rates both the monitoring plan and surveillance testing did not show a significant variation because of the very low violation rates. Pesticides were not found in any samples of domestic products. Residue violations in cattle and pork mainly represented in penicillin, quinolone and aminoglycoside classes. Upon investigation of causes for residue violation, the safety regulations were not performed as follows: compliance with the inappropriate withdrawal period for drugs after administration, giving a drug by mistake and mismanagement of water supply.

Conclusions: The NRP monitoring would provide the decrease of risk for chemical residues in foods of animal origin and fundamental scientific background to ensure food safety.