the frequency of $CD8^+$ and $CD3^+$ cells slightly increased eight hpi with Group 1 viruses as well as with strain 42. Those cells also produced more IFN-γ and TNF-α, and IL-10 secretion was significantly downregulated in cell supernatant infected with Group 1 viruses compared with Group 2 viruses. However, each PRRSV did not influence the level of other cytokines (IL-6 and IL-12). CD3 expression analysis suggested stronger T-cell activation in Group 1 infection.

Conclusions: This study showed that serum neutralizing antibody titer among viruses was related to certain cytokines, suggesting that those cytokines could contribute to customized vaccine development.

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Outbreak of avian botulism of quail in Korea

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Introduction: Avian botulism is a serious paralytic disease of birdscaused by botulinum neurotoxin (BoNT) that is produced by *Clostridiumbotulinum*. Most cases of animal botulism in poultry and wild birdsare caused by BoNT types C and D. Wereport the outbroken of avian botulism in farming quails in Hoengseong-gun,Gangwon-do in July, 2016.

Materials and Methods: The liver samples of quail were incubated at 70℃ for 15min, and cultured using TPGY (Trypticase Peptone Glucose Yeast Extract) in 37℃ with anaerobic condition. Forthe detection of toxin genes, DNA was extracted using the culture supernatant after2 days incubation. The toxin gene was detected by the single tube nested PCR. Forthe isolation of *Clostridium botulinum*,the supernatant was sub-culturedusing McClung toabe agar for 3 days at 37℃ with anaerobic condition. Wedetermined the causative toxin type of *Clostridiumbotulinum* using mouse bioassay with filtered supernatant inoculums and severaltypes of antitoxins.

Results: The toxin geneof *Clostridium botulinum* was detectedby single tube nested PCR in the quail's liver samples and we isolated the *Clostridium botulinum*. Mouse bioassayshowed that the supernatant was neutralized with *Clostridium botulinum* type C antitoxin, thus the mice did not showany clinical signs of botulism. In the mouse bioassay, the mice did not showany clinical signs of botulism with *Clostridiumbotulinum* type C antitoxin. As a result of detection of toxin gene,isolation of *Clostridium botulinum*and confirmation of toxin type, we diagnosed as avian botulism caused by typeof toxin C.

Conclusions: The outbroken of avianbotulism in a quail farm is the first case in Korea. In general, the avianbotulism had been broken in wild birds or from the environment including soilof poultry farm. Unusually, this case was found in cages of quail farm.Therefore, the cause of the broken of avian botulism in quail is considered to bequail's farming environments including cage, but the exact cause was notelucidated. So, the outbroken of avian botulism in quail should be monitoredcarefully containing farming environment.

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The Occurrence of Bacterial Chondronecrosis with Osteomyelitis of Broiler Chickens in Korea from 2012 to 2015

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Introduction: Lameness compromises the welfare of chickens and causes considerable economic loss. Lame birds cause the difficulty in accessing food and water, and eventually cause dehydration and death. Osteomyelitis was first reported as a cause of lameness in commercial broiler chickens in Australia, and *Staphylococcus aureus* was shown to be the cause. The bacterial chondronecrosis with osteomyelitis has subsequently been reported in broilers from Australia, US, Canada and Europe. We report the occurrence of bacterial chondronecrosis with osteomyelitis of broiler chickens in Korea from 2012 to 2015.

Materials and Methods: We performed observation of clinical sings and postmortem examination from diagnostic cases of the broilers. And also performed bacteriological and histopathological exam. We finally identified the cultured bacteria from lesions with VITEK Identification System.

Results: We diagnosed as chondronecrosis with osteomyelitis from submitted clinical cases of broilers 2012 to May, 2015. The occurrence rate of chondronecrosis with osteomyelitis was 0.6~13% from totally 30 diagnostic cases. The age of cases was 2~19-old day and clinical signs were lameness and stretched one-side leg. In postmortem examinations, fracture of femoral head and desquamation of cartilage were observed. In the histopathological examination, inflammation of cartilage in the femoral head was observed. And also osteomyelitis and epicarditis was observed when infected with *Enterococcus cecorum*. In bacteriological examinations, three major bacteria species were involved. There were *E. coli*,

Staphylococcus sp. and *Enterococcus sp*. Total 46 cases of specimens, *E. coli* was isolated as 57%, *Staphylococcus sp*. was 48% and *Enterococcus sp*. was 39%. In the same specimen, two or three kinds of bacteria also was even isolated. The *Staphylococcus sp*. was comprised by 8 kinds of species including *S. cohnii* main isolate. The *Enterococcus sp*. was comprised by 4 kinds of species including *E. faecalis* main isolate.

Conclusions: Three kinds of bacteria were involved in occurrence of chondronecrosis with osteomyelitis in broiler. The major isolated bacteria was *E. coli*, but we estimated the *E. coli* infection was subsequently infected bacteria after other bacteria or virus invaded and the condition of the body was lowered. Although *E. coli* was main isolate, we think the main causes of chondronecrosis with osteomyelitis are *Staphylococcus sp*. and *Enterococcus sp*. The exact role of the *Staphylococcus sp*. and *Enterococcus sp*. in the occurrence of chondronecrosis with osteomyelitis will be studied in near future.

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Evaluation of antiviral efficacy of synthetic natural products against PRRSV replication

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Introduction: Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most economically important infectious diseases of swine worldwide caused by PRRSV. Until today, the control strategies of PRRS are unsuccessful due to in effective vaccines efficacy or unavailability of effective anti-PRRSV drugs. Modified live virus vaccines (MLV) are widely practiced though there is serious concern of using MLV as vaccine virus reverted to virulence reported by many previous studies. Antiviral therapeutics is an alternative or additional control measures to combat the virus infections; wherever, if there are no successful vaccines to match well with the circulating virus. Therefore, a pharmacological intervention might be a suitable and appreciated alternative control strategy in PRRSV infections. Many previous studies have been reported that a number of natural compounds and compositions proved their antiviral activities against a number of viruses. Thus, the present study was aimed to evaluate antiviral efficacy on ten synthetic natural products against PRRSV replication in cells culture system.

Materials and Methods: VR2332, a prototype strain of North American PRRSV was used in the present study. Ten natural products (CMA-2-15, CMA-2-150, CMA-2-152, CMA-2-153, JJH-5-136, JJH-5-147, JJH-6-14, JJH-6-33, 93PSY-2-13 and 93PSY-3-31) were preliminary screened to evaluate their antiviral efficacy on PRRSV replication in MARC-145 cells with four different concentrations (0, 10, 20 and 40 μM). Confluent monolayer of MARC-145 cells was prepared in 12-well plates and treated with growth medium (RPMI-1640) containing the concentrations (0, 10, 20 and 40 μM) of each reagent for 2 hours at 37℃ and 5% CO2 in a humidified chamber. After incubation, the supernatant was discarded, and cells were inoculated with VR2332 at a MOI of 0.001 and incubated for 1 hour. After 1 hour incubation, virus inoculum was discarded and cells were replenished with growth medium containing the same concentrations of each reagent. Then, the treated plates were incubated for 4 more days. Supernatants were collected from each plate at every 24 hours, and the progeny virus titers were measured by micro-titration assay. Virus titers was calculated based on cyto-pathic effect (CPE) and was expressed as a 50% tissue culture infective dose ($TCID_{50}$/ml). Consistently, based on preliminary screening results, JJH-6-33 and 93PSY-3-31 were evaluated to demonstrate their final effective concentrations on PRRSV replication in PAMs and MARC-145 cells at six different concentrations (0, 10, 20,40, 60 and 80μM) following the same method described above. In addition, cytotoxicity of JJH-6-33 and 93PSY-3-31 was also measured in PAMs and MARC-145 cells using MTT assay with the same concentrations used in antiviral assays.

Results: JJH-6-33 and 93PSY-3-31 were significantly effective on PRRSV replication among all the ten natural products. VR2332 replication was significantly decreased in PAMs and MARC-145 cells approximately by 3-folds or 6-folds lower in the presence of JJH-6-33 at the concentrations of 10or 60μM, and viral growth was completely suppressed in the presence of 80μM without showing significant toxicity in both cells up to 48 hours posttreatment (hpt). Similarly, 93PSY-3-31 significantly suppressed VR2332 replication approximately by 3-foldsor 6-folds lower in the presence of 10or 80μM concentrations in PAMs without causing high cytotoxicity. However, 93PSY-3-31 significantly decreased VR2332 replication in MARC-145 cells in dose-dependent way with the concentrations of 40μM or higher without showing high toxicity, though the lower concentrations (10 and 20μM) did not suppress virus replication.

Conclusions: JJH-6-33and 93PSY-3-31 potentially inhibited PRRSV replication in cells, suggesting that both compounds might be good candidates for therapeutic agents against PRRSV infection though their efficacy and safety need to be evaluated based on in vivo assessment.

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