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P-018

Application of Multilocus Sequence Typing to *Mycoplasma hyorhinis* Strains Isolated in Korea

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Introduction: *Mycoplasma hyorhinis* is associated with a number of swine diseases such as polyserositis and Enzootic pneumonia, resulting in retarded growth and reduced feed efficiency. Despite of such etiological significance, epidemiological study on *M. hyorhinis* circulating in the swine population is still somewhat scarce. Meanwhile, multilocus sequence typing (MLST) method is widely used as a powerful tool for investing the population structure of various bacterial species including mycoplasma. In this study, population genetics among *M. hyorhinis* strains prevalent in Korea was analyzed by a highly discriminatory MLST method described previously[1].

Materials and Methods: Isolation and cultivation procedures of mycoplasma were performed by the method described by Friis[2]. In detail, lung tissues were collected from the pigs derived from different geographic origins at slaughter houses. *Mycoplasma hyorhinis* strains were identified and confirmed by a multiplex PCR[3]. Genetic relationship among the isolates was determined by MLST method on six housekeeping genes; *dnaA*, *rpoB*, *gyrB*, *gltX*, *adk* and *gmk*. To amplify the target gene fragments, PCR was performed on the genomic DNA of each isolates with a pair of primers of each gene. After running agarose gel, the PCR products were extracted using MEGAquick-spin total fragment DNA purification kit (iNtRON, Sungnam) and were directly sequenced (Bioneer, Daejeon). A comparative analysis of MLST on the genes was carried out by BIGSdb, written by Keith Jolley[4].

Results: Thirty-eight field strains of *M. hyorhinis* were obtained from different farms in Korea. Sequence types (STs) of the isolates were analyzed by online database for the *M. hyorhinis* MLST scheme (<http://pubmlst.org/mhyorhinis/>). Genetic variation ranges from one (*adk*) to four (*dnaA* and *gyrB*) as the number of allelic at the different loci. Overall, the isolates were classified into nine types out of 29 STs described

in the previous study[1], and the allelic profile of 114111 was a major ST, covering 14 out of 38 isolates.

Conclusions: We collected *M. hyorhinis* field strains from swine farms of various geographic origins in Korea and determined their sequence types by MLST method. This result indicates that *M. hyorhinis* strains prevalent in Korea can be grouped on the basis of genetic variation and the MLST method would pave for the way of epidemiological investigations on *M. hyorhinis* infections.

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P-019

Effects of Calcium on the Formation of Pre-neoplastic Lesions in the Colon of Mice

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Introduction: The beneficial actions of calcium (Ca) on the colonic epithelium have been demonstrated with recent insights focusing on its function in preventing neoplastic transformation. Calcium exerts anti-proliferative effects on cellular targets through the promotion of differentiation and apoptosis. We investigated the influence of calcium on the formation of colonic aberrant crypt foci (ACF), as induced by azoxy methane (AOM) followed by dextran sodium sulfate (DSS) in ICR mice.

Materials and Methods: Six-week old ICR mice were received three (0~2nd weeks) i.p. injections of AOM (10 mg/kg B.W), followed by 2% DSS with drinking water for a week to induce ACF as pre-neoplastic lesions. There were 4 experimental groups including normal group and control (AOM/DSS) group, AOM/DSS + 1.0% Ca group, AOM/DSS+ 2.0% Ca group. Calcium (1.0 or 2.0%) was treated with drinking water for 12 weeks. After sacrifice the total numbers of aberrant crypt (AC) and aberrant crypt foci (ACF) were measured in the colonic mucosa after methylene blue staining.

Results: Control group showed the number of 11.58 ± 2.43 ACF/colon, which were composed of a total number of 30.42

± 5.18 AC/colon. The number of ACF with more than 3 ACs was 2.37 ± 0.68, which is likely to advance to colon cancer. As compared to the control, the calcium treatments at 1.0 or 2.0% significantly decreased the number of total ACF and AC in a dose-dependent manner. The ACF number with more than 3 ACs also significantly decreased in calcium treatment groups compared with control group. The decrease in ACF or AC by calcium treatment was associated with a decrease in cell proliferation and an increase in apoptosis in colonic mucosal cells.

Conclusions: These results suggest calcium may exert a protective effect against colon cancer by inhibiting the development of ACF/AC in ICR mice.

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P-020

Preventive Effects of OxC-beta on a Necrotic Enteritis Challenge Model of Broiler Chickens

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Introduction: Beta-carotene is one of the most abundant carotenoid compounds and may contain beta-carotene derived oxygen copolymers (OxC-beta). In vivo assays indicate Beta-carotene treatment enhances the phagocytic activity of monocytes and increases the intestinal immune function. Necrotic enteritis (NE) has been widely found in the commercial broiler chickens since the ban of antimicrobial growth promoters. This study was evaluated the preventive effect of OxC-beta (OxC-beta™ Livestock product from Avivagen Inc., Canada) in a subclinical necrotic enteritis broiler chicken model with various items, including survival (mortality) rate, clinical sign, body weight, weight gain, intestinal lesion, and bacterial enumeration compared to the non-medicated bird group.

Materials and Methods: Animals used were vaccinated (Newcastle disease virus) Ross broiler chicks obtained at 1 day old from a commercial hatchery. The evaluation items for all birds were carried out in the chicken isolator during the whole experimental period. Non-antibiotic and non-anticoagulant broiler feed was supplied to the bird groups. For the subclinical necrotic enteritis chicken model, *C. perfringens* (CP-13) isolated from broiler chickens with necrotic enteritis was challenged orally two times a day with approximately 1×10^8 CFU/ml

during the 14-to-16 day periods.

Results: Since the *Clostridium perfringens* challenge, mean body weight of the OxC-beta treatment groups was significantly ($P < 0.05$) increased relative to the non-medicated bird group. The body weight of the 2 parts-per-million (ppm) OxC-beta treatment group steadily increased for the 1-to-28 day period. The average weight gain of OxC-beta treatment groups for the whole trial period was restored to the weight achieved by the non-challenged bird group. The intestinal lesion scores due to NE infection were significantly ($P < 0.05$) alleviated by OxC-beta treatment groups compared to the non-medicated bird group. Among them, the lesions of the 2 ppm OxC-beta treatment group was more improved than the remaining treatment groups. The number of Clostridial bacteria in feces was reduced by OxC-beta with a dose-dependent pattern.

Conclusions: Low ppm levels of OxC-beta in feed can contribute to the prevention and improvement of NE in commercial broiler chicken farming and is expected to have a positive effect in improving productivity for the feeding period. This research was supported by Avivagen (Avivagen Inc., Ottawa, Canada), Project title: "Preventive effect of OxC-beta on Necrotic Enteritis Model with Broiler Chicken". This research was supported by Animal Disease Management Technology Development (Project No. : 311007-5), Ministry of Agriculture, Food and Rural Affairs.

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P-021

Development of Biomarkers Utilizing Variable Lymphocyte Receptors (VLRs) of Inshore Hagfish (*Eptatretus burgeri*) against Avian Influenza Virus

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Introduction: Hagfish along with lampreys are jawless