

primed with LPS for 3 h. After LPS priming, cells were replaced by RPMI 1640 containing the inflammasome activators in the presence of RGE, saponin fraction, and non-saponin fraction.

Results: In this study, we attested the roles of RGE, saponin fraction (SF), and non-saponin fraction (NS) on the priming or the activation of inflammasomes using murine bone marrow-derived macrophages. As results NS and SF differently regulated the two steps of inflammasome. That is, NS induced pro-inflammatory cytokines in the priming while it did not alter the activating step. Conversely SF acted as an inhibitor at the both steps. The promoting effects of NS on cytokine expression were further confirmed in mice.

Conclusions: Thus saponin and non-saponin of RGE differently regulates the signaling of inflammasome.

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Characterization of HN genes of avian paramyxovirus type 4 isolates

Chan-Yoon Bae¹, Haan-Woo Sung¹, Hyuk-Moo Kwon¹, Hyun-Jeong Lee², Ji-Ye Kim², Kang-Seuk Choi², Min-Jun Jang¹

¹College of Veterinary Medicine, Kangwon National University, Chuncheon 200-701, South Korea; ²Avian Diseases Division, Animal and Plant Quarantine Agency, Anyang 430-757, South Korea

Introduction: Avian paramyxoviruses (APMV), members of the family *Paramyxoviridae*, are single-stranded, nonsegmented, negative-sense RNA viruses. APMVs are classified into 12 serotypes (APMV-1 to 12) based on their antigenicity of hemagglutinin. Among them, APMV-1 which is known as the Newcastle disease virus causes severe disease in poultry. Apart from the well-characterized serotype APMV-1, the genetic diversity of other APMV serotypes was relatively unknown. Although there were many reports of APMV-4 isolates, the information of APMV-4 genomic sequence provided was limited. In this study we describe the genetic diversity of APMV-4 isolated from wild birds.

Materials and Methods: During 2009 to 2014, APMV-4 viruses were isolated from fecal droppings of wild birds. All APMV-4 isolates were isolated by chicken egg inoculation and confirmed by a conventional hemagglutination inhibition test with a reference paramyxovirus antiserum panel. The full hemagglutinin-neuraminidase (HN) gene was sequenced and analyzed to compare the diversity of APMV-4. Viral RNA was extracted from infected allantoic fluid by using Viral Gene-Spin viral DNA/RNA extraction kit (iNtRON Biotechnology, South Korea). Standard reverse transcription-PCR was performed by use of a one-Step RT-PCR kit (iNtRON Biotechnology, South Korea) with various APMV-4 specific primers. Sequencing was performed with DNA sequencing service in Macrogen Inc (Seoul, Korea). Sequence data obtained in this study were aligned with Mega 6 Clustal W method. Phylogenetic trees were generated by the neighbor-joining method using the MEGA 6.06 software with 1000 bootstrap replications.

Results: Six APMV-4 viruses isolated from various wild birds (two isolates from mallard, one isolate from spot-billed duck, one isolate from gray heron and the other two isolates from unknown wild birds) were characterized in this study. The HN gene is 1,914 nt long for all of the APMV-4 strains. The HN protein of Delaware (APMV-4/duck/Delaware/549227/2010) has a length of 569 amino acid (aa), which is identical to HN protein length in strain from Korea (APMV-4/KR/YJ/06) and Hong Kong (APMV-4/duck/Hongkong/D3/75 complete genome.); the HN proteins of the Belgian (APMV-4/mallard/Belgium/15129/07) and Italian strains (APMV-4 strain IT-3670, 3936, 4103, 4284 and 4532) are 565 aa long. Critical sequence features like potential glycosylation sites, putative sialic acid binding motif (NRKSCS), neuraminidase active residues and cysteine residues are conserved among all of the APMV-4 strains. The nucleotide sequence homology of HN gene within the isolates was variable values, ranged from 97.5% to 99.1%. Phylogenetic trees based on the nucleotide sequences of the HN proteins of the six isolates of APMV-4 showed that six isolates could be clearly divided into two classes.

Conclusions: Phylogenetic analysis of the HN gene of the six isolates of APMV-4 showed that Korean isolates were divided into two classes; class 1, class 2. Two strains (APMV-4/mallard/KR/KNU53/2012, APMV-4/wildbird/KR/KNU 55/2013) categorized as class 2 were more closely related to the Italian strains (APMV-4 strain IT-3670, 4103) than the other four strains. The other four strains (APMV-4/wild bird/KR/KNU 9/2009, APMV-4/spot-billed duck/KR/KNU 23/2011, APMV-4/mallard/KR/KNU 82/2013 and APMV-4/gray heron/KR/KNU85/2014) were categorized as class 1 with Korean strain (APMV-4/KR/YJ/06). These results suggested that APMV-4 strains have genetic diversity and are needed to further define the antigenic or pathogenic diversity.

References

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Application of *Ecklonia cava* Kjellman by-product as a feed additive: Enhancing weight gain, immunity and protection from *Salmonella* infection in chickens

Chung Yoh Kim¹, Soyeon Park¹, Kiju Kim¹, Han Jong Keon², Tae-Wook Hahn¹

¹College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 200-101, Republic of Korea; ²Milae Resources ML Co. Ltd., Ogeum-ro 11 gil, Songpa-gu, Seoul 138-8277, Republic of Korea