According to the phylogenetic tree analysisbased on the nucleotide sequences of S1, S2, M and N genes of IBV K037-12isolate, S1, S2 and M genes of its were clustered into CK, CH, IBTZ, 2012 group and N gene of its was clustered into KM91 group. This study showedthat recombination events occurred between IBVs and vaccine strains. This studywill be useful for the control of IBV in Korea.

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P-016

Sequence analysis of canine and feline coronaviruses circulating in Korea

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Introduction: The infection of canine (CCoV) and feline (FCoV) coronavirus is common in dogs and cats, especially in young age. CCov and FCoV are mostly asymptomatic or only cause mild enteric disease. Coronavirus (CoVs) is member of the order *Nidovirales*, family. Coronaviruses are enveloped and have a large (27-32kb) single-stranded, positive-sense RNA. However CoV can very easily be mutated or undergo recombination and result in fatal disease. The two thirds of CoV genome encodes a non-structural polyprotein and the rest encodes structural proteins, which consists of spike (S), membrane (M), envelop (E), and nucleocapsid (N). In this study, the sequences of CCoV and FCoV from samples obtained dogs and cats around Seoul and Chuncheon in 2014 and 2015 year were determined and compared with those of past Korean and non-Korean CCoV and FCoV strains.

Materials and Methods: CCoVs and FCoVs were detected from fecal samples of dogs and cats in Seoul and Chuncheon. Fecal samples were collected from individual dogs and cats by 6 inch cotton tipped applicators and suspended in 1ml phosphate buffer saline. The collected samples were then centrifuged at 1500 rpm for 10 minutes and supernatants were used in Viral Gen-spin Viral DNA/RNA Extraction kit to extract the RNAs. Primers were synthesized based on the published CCoV, FCoV sequences and used to amplify the partial M and S genes. RT-PCRs were performed with a One-Step RT-PCR Kit using respective primer pairs. The Nested PCR was performed with Maxime PCR Premix with those sample that could not be identified by RT-PCR. PCR products of the correct size were purified from 1% agarose gel using a QIAquick Gel Extraction Kit. PCR products were cloned into the pCR2.1-TOPO vector using a TOPO TA Cloning Kit. Positive clones were amplified in LB broth for 24 hours. Amplified clones were extracted plasmid DNAs by DNA-spin Plasmid DNA Purification Kit. Plasmid DNAs were sequenced in both directions by fluorescence-based sequencing. The phylogenetic and molecular evolutionary analyses were conducted using the Lasergene version 12 (DNASTAR, Inc) computer program.

Results: In the partial M gene, Korean CCoV isolates shared approximately 89.3-100% nucleotide sequence identities with each other, 86.3-96.7% nucleotide sequence identities with Korean CCoVs isolated in 2012 and 77.4-99.5% nucleotide sequence identities with non-Korean CCoV strains. As for partial S gene, Korean CCoV isolates had approximately 64.6-96.7% nucleotide sequence identities with each other and 63.5-99.7% nucleotide sequence identities with non-Korean CCoV strains. Phylogenetic analysis based on the partial M gene region showed two distinct genetic clusters, type I and type II. Six Korean CCoVs belonged to first cluster (type II) along with the CCoV reference strain BGF10 (CCoV2a, UK). All of the six Korean CCoVs formed the independent subcluster. The other five Korean CCoV isolates were grouped into the second cluster (type I) along with the FCoV-like CCoV 259/01(ITL) strain. Two FCoV isolates were closely related to reference strain FCoV-89/1683 which belonged to FCoV Type II. Phylogenetic analysis based on partial S gene revealed that there were basically two distinct genetic clusters just same as M gene. Three Korean CCoVs were grouped into first cluster (type II) which was closely related to the reference strains CCoV-1/71(CHN), FCoV 79-1146(NED), and 79-1168(UK). The other sample was grouped into the type I along with Italian CCoV 23/03 and Elmo-02 strains. Phylogenetic trees based on the partial M gene and S gene showed high similarity with each other.

Conclusions: Nucleotide alignment and phylogenic analysis of the partial M and S gene sequences showed that at least two CCoV genotypes were circulated in dogs in Korea. The genotype 2 cluster turned out to be a distinct cluster that is different from those circulated before 2012. Almost half of the CCoVs turned out to be genotype 1 which is the most distinguishable change in 2014-2015. Two FCoVs belong to genotype 2 cluster.

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P-017

Genetic identification and sequencing analysisof non-primate hepaciviruses from horses in Korea

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Introduction: The genus *Hepacivirus*, one of the four genera in the family *Flaviviridae*, includes the hepatitis C virus (HCV) andGB virus B (GBV-B). Hepatitis C virus (HCV), the major causative agent ofchronic hepatitis, has a restricted host range. Whilst higher primates aresusceptible to experimental infection, natural infection has only been detectedin humans. An estimated 3% of the world population is chronically infected withHCV, which is causally linked to cirrhosis, liver failure and hepatocellularcarcinoma. In 2011, Kapoor et al. describeda novel virus with considerable genomic similarity to HCV in respiratorysamples of domestic dogs and tentatively named it canine hepacivirus. Accordingto the recent studies, hepaciviruses have also been detected in horse serum, bats,and wild rodents. These viruses are currently grouped as non-primatehepacivirus (NPHV). Comparative phylogenetic analysis confirmed NPHV as theclosest genetic relative of HCV. The distribution of NPHV among horses has beenreported in limited geographical areas including the UK, Germany, the USA, Brazil,and Japan. However, NPHV has not reported in Korea yet. In this study, we detectedand seqenced non-primate hepaciviruses from horses in Korea.

Materials and Methods: Serum samples were obtainedfrom horses, showing non clinical signs, in 2015 in Chuncheon-city, Gangwonprovince, Korea. Viral RNAs were extracted and purified with NucleoSpin RNA Plus. Primers were synthesized based on thepublished NPHV sequences and used to amplify the NS3 serine protease helicasegene. RNAs were used for RT-PCR with a One-Step RT-PCR Kit using respectiveprimer pairs.PCR products were also amplified by nested-PCR with Maxime PCR Premix Kit usingrespective nested primer pairs. PCR products of the correct size were purifiedfrom 2% agarose gel using a Fragment DNA Purification Kit. Eluted PCR products were cloned into the pCR2.1-TOPO vectorusing a TOPO TA Cloning Kit. Positive clones were extracted plasmid DNAs byDNA-spin Plasmid DNA Purification Kit. Plasmid DNAs were sequenced in bothdirections by fluorescence-based sequencing. The NS3 sequences were assembledand analyzed using the Lasergene version 12 (DNASTAR, Inc) computer program.

Results: Serum samples were tested for the presence of NPHV using the NS3 nestedPCR. Positive samples were detected and confirmed as NPHV by sequence analysis. Sequence comparison analysis demonstrated that the genomic nucleotides identitybetween Korean NPHVs was 96.6%. The nucleotide identity between Korean NPHV andother NPHV from horses was 84.2%-97.0%, and between Korean NPHV from horses andother NPHV from dogs was 85.4%-86.4%, and between Korean NPHV from horses andhuman hepatitis C virus was 58.1%-63.4%, and between Korean NPHV from horsesand other NPHV from rodents was 57.0%-61.1%, and between Korean NPHV fromhorses and other NPHV from bats was 55.8%-59.6%. According to phylogenetic treebased on nucleotide sequence of partial NS3, Korean NPHV strains closelyrelated with each other and strains from USA (JQ434008 and KJ472766).

Conclusions: The results from this study identified thepresence of NPHV from horses in Korea for the first time. Further investigationof genetic and serological surveillance and characteristics of NHPV fromdiverse mammals in Korea is needed.

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P-018

Experimental infection of highly pathogenicavian influenza virus subtype H5N8 in wild birds

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Introduction: Highly pathogenic avian influenza virus (HPAI)outbreaks cause serious financial damage to poultry industry and raise publichealth concerns. Recently, several HPAI outbreaks caused by H5N1 have beenreported. From 2014, there have been more incidences of H5N8 subtype outbreaksand draw public attention. The study aims to determine infectivity andmortality as well as infection patterns of H5N8 HPAI in wild birds.

Materials and Methods: Mallard ducks, Mandarin ducks and pigeons (n=6each) were infected with H5N8 virus (2×10^6 EID_{50}/dose)via nasal cavity in ABL3 facility in Konkuk University. Clinical signs andmortality were recorded for 2 weeks. Tissue samples were prepared using routinehistological process. Immunohistochemical detection of H5N8 virus was carriedout to determine distribution of the virus.

Results: H5N8 HPAI showed low infectivity in challengedand exposed Mandarin ducks (0/6) and the same were observed in pigeons (0/6).However, high infectivity was observed in Mallard ducks (6/6). Morbidity andmortality were low in all animals tested as only 1 Mandarin duck was infectedby contact exposure and died within 4 days. Immunohistochemical study showedfrequent viral antigen detection in respiratory and digestive system as well ascecal tonsil and heart.

Conclusions: Our study shows that wild birds are infectedwith H5N8 without showing clinical symptoms thus may play important roles inH5N8 spreading. Especially Mallard ducks would serve as efficient carriers asthey showed high infectivity but low mortality.